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MADINAT AL-HIKMAH

City of Education, Science and Culture

Shaheed Hakim Mohammed Said (1920-1998), a scion of the renowned South Asian Hamdard family, decided in 1948 to make the newly created Pakistan his home. He settled down in Karachi and by untiring, single minded devotion and commitment, braving all handicaps, created Hamdard Pakistan. He developed it into the leading pharmaceutical complex of Eastern Medicine in the country, run on the latest modern lines and techniques, supplying drugs of high quality and purity, backed with free clinical consultations to help ailing humanity. Hamdard Pakistan, under his leadership, also emerged as the leading philanthropic organization, and also tried to motivate people through dialogue, conferences, and journals like the *Hamdard Medicus*. In addition, he provided help to various institutions and academic bodies.

He was restless to do more, and during one of his *Hajj* pilgrimages, he envisioned the creation of a comprehensive City of Education, Science and Culture: the Madinat al-Hikmah. Work on it was initiated and funded by the Hamdard Foundation Pakistan in 1981, in the picturesque surroundings of Bund Murad Khan, 35 kilometers away from the city centre. Today it is a beehive of activity, with children and youth engaged in academic, technical and sports activities, devoted to promoting learning and culture, and through it, help achieve moral and physical welfare, peace and progress. The Bait al-Hikmah Library, Hamdard Public School, free Hamdard Village School, Centre for Horticulture and the Hamdard University are the major institutions found here. The Hamdard University's mission provides value-based education to all students in its constituent institutions. Its prestigious institutions – some based at the main campus and some in the city and some in Islamabad - include Hamdard Institute of Management Sciences (HIMS), Hamdard College of Medicine and Dentistry (HCMD), Hamdard Al-Majeed College of Eastern Medicine (HACEM), Shifa ul-Mulk Memorial Hospital, Hamdard University Hospital – Naimat Begum Mother & Child Care Unit (part of the Hamdard University Centre of Excellence), Hamdard Institute of Education and Social Sciences (HIESS), Dr Hafiz Mohammad Ilyas Institute of Pharmacology and Herbal Sciences (HMI-IPHS), Hamdard School of Law, Faculty of Pharmacy and Faculty of Engineering Sciences and Technology (FEST). The Madinat al-Hikmah continues to develop and grow.

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The *HAMDARD MEDICUS* since 58 years has been publishing original articles, reviews, short communications, history of traditional medicine and case reports on all aspects of complementary medicine and pharmaceutical sciences in English. Manuscripts to *Hamdard Medicus* are accepted for consideration with the understanding that the findings have not been published earlier.

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Times New Roman 12-point is recommended for text, tables and figures. All text should be written in the third person. The sections includes: Title page, abstract, text, references, tables, list of titles for all figures (typed on one page), and figures. The page numbers of the entire manuscript should be at the bottom right corner of each page.

Title Page Should Contain: Title of the manuscript, Short title, Authors names with superscripts representing their affiliation clearly marked *corresponding author with Telephone, Fax and E-mail address.

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The Text For Articles Should Contain: Main headings in caps and bold as:

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Clinical Efficacy and Safety of Cupping Therapy

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Abstract

In Unani System of Medicine, since centuries cupping therapy (Al Hijamah) has been an integral part of regimental therapy to treat various human diseases. The present study focuses on the therapeutic effect of wet cupping therapy (combination of suction and controlled medicinal bleeding) against various diseases. It is the oldest form of alternative medicine in which a suction is created locally on the skin, physicians consider that this act trigger blood flow in order to support healing process. The patients (n = 65) aged between 20-65 years, enrolled in this study after obtaining their informed consents at Eastern Specialist Clinic, Karachi. They were clinically assessed and diagnosed on the basis of their past history, clinical observation and temperamental assessment grouped into: chronic constipation, dyspepsia, knee joint pain/arthritis, migraine, shoulder pain/muscular spasm/cervical pain, sciatica, stress/anxiety/depression and varicose vein. The cupping therapy was applied on the back and front of the body over different points (5-6) for a period of 3-5 minutes comprising 3-10 sessions with an interval of 15 days. The wet cupping was effective in 72% of patients which showed improvement in their corresponding symptoms in order of chronic constipation > migraine > sciatica > shoulder/cervical/muscular pain > varicose veins > dyspepsia > knee joints pain > stress/anxiety/

depression. The relief from symptoms depended on temperamental evaluation and its effectiveness appears to be: Sanguineous temperament *harr* (hot) and *ratb* (moist) > phlegmatic temperament *barid* (cold) and *ratb* (moist) > bilious temperament *harr* (hot) and *yabis* (dry) > melancholic temperament, *barid* (cold) and *yabis* (dry).

It is concluded that wet cupping therapy is effective against diseases of various origins and its efficacy is dependent on temperament and severity of the disease.

Keywords:

Unani system of medicine, Wet cupping therapy, Temperament.

1. INTRODUCTION

The historical background of cupping therapy seems prominent to ancient Egyptian, Chinese, and Middle Eastern cultures as indicated in the oldest medical textbooks in the world, the *Ebers Papyrus*, illustrates its practice during 1550 B.C by Egyptians to remove by bleeding the foreign matter from the body and triggering blood flow thus encouraging the curative process of various medical ailments. Hippocrates (Father of Modern Medicine) and Galen were also enormous supporters of cupping (Ahmad and Qadeer, 1998). Unani System of Medicine is based upon the *Nazariyya-i-Akhlat* (theory of humors) as proposed by Hippocrates emphasizing the presence of four humours in

the body, namely *dam* (blood), *balgham* (phlegm), *saфра* (yellow bile) and *sawda* (black bile) (Wasti, 1990).

Each humor is associated with specific temperaments i.e. blood is *harr* (hot) and *ratb* (moist), phlegm, *barid* (cold) and *ratb* (moist); yellow bile, *harr* (hot) and *yabis* (dry); black bile, *barid* (cold) and *yabis* (dry). Any imbalance in the humoral levels, if not controlled by innate system may lead to the development of various diseases (Ahmed, 2009).

The concept of temperament characterizes the individuality of a human being (Rolfe, 2002) reflecting communal measure of an individual corporal structure and psychosomatic profile or personality. The above theory has laid a strong basis as its origin reflects back to centuries and is still applied with suitable adaptations concerning different medical and scientific spheres (Azmi, 1995). As temperament is the amalgamation of physical, psychological, emotional and spiritual features, depending on these specific qualities there are four types of temperaments: Sanguinous (with qualities of Hot and Moist) Phlegmatic (with qualities of Cold and Moist) Bilious (with qualities of Hot and Dry) Melancholic (with qualities of Cold and Dry). However, each individual has trait from all four temperaments with a dominance of one of the temperament types. Having a unique temperament a combination of qualities exists in an individual. That's why there is a difference in people experiencing either cold or hotness in a unique way depending on the individual temperament. Predispositions to illnesses as well as pathological and therapeutic processes may result from the strong impact of temperament and associated qualities of a person. Qualities associated with the temperament of the individual play an important role in the predisposition to specific illnesses, this concept in Unani-Tibb is employed not only to the treated and to the

sufferer's but to the disorder affecting or even to the type of prescription or activity suggested as therapy. It is stated in Unani-Tibb that individuality of every patient should be considered during treatment (Bhikha and Haq, 2000).

In order to release toxins and unwanted matter from the tissues and organs, cupping is considered to be the oldest and most effective methods. Cupping can be classified as: fire cupping, massage cupping and cupping by horn method (Anjum *et al.*, 2005). Other than that mainly there are two classes of cupping: 1) dry cupping and 2) wet cupping. In dry cupping therapy the skin directly underneath the cup is sucked upwards by creating a vacuum within the cup and hold for 5-10 minutes while in wet cupping therapy superficial cuts are made on the skin below the cup lightly lacerated enabling the blood to be drawn from the skin within the cup (Li *et al.*, 2006). A modified version of cupping therapy is massage cupping that has been used broadly in Chinese medicine. In this system suction and negative pressure is created along with massage to bring blood flow to stagnant muscles and skin, loosen adhesions, connective tissue and stubborn knots in soft tissues, drain excessive fluids and toxins, to stimulate the peripheral nervous system. Before the application of the cup, preferably olive oil is applied to the skin, after identifying the region of tenderness and blockage in order to make possible smooth movement. The vacuum is created by placing the cup on to the affected areas followed by gently gliding it on that area. It is placed around 5 mins on persistent tender areas and on swollen joints as well as tissues. Strong massage cupping leads to reddened skin, signifying blood movement towards the surface and ready for the application of liniments of natural origin (preferably sunflower, linseed, energy producing castor oil and olive oil), analgesics (rose oil, babuna oil), plant hydrosols

(Rose, Roman Chamomile, Neroli and Lavender) or essential oils (Rose, Roman Chamomile, Jasmine and Lavender) (Anita J. Shannon, A.C.E., Massage Cupping, www.massagecupping.com) immediately facilitating absorption deeper into the tissue for relieve of pain and obstructions. Local increase in blood flow nourishes the skin and muscles thereby, allowing contaminated matter to be removed from the nearby tissues and organs towards the surface to be eliminated. In case of wet cupping therapy, incisions are made in order to remove blood which has been brought up just beneath the surface of the skin. The concept of cupping is to let out bad blood that is supposed to be injurious to the body leading to improve in the flow of life energy, which travels throughout the body in channels called meridians (Arzani 1940).

The basic philosophy behind cupping is that both inflammation(s) and pressure(s) are diverted from the major organs including brain, heart, liver, lungs, and kidneys to the skin. It assists in the healing process by supporting the immune system, hence resulting in the optimal performance of the body. It also aids the actions of physis, ward off toxins and other injurious impurities from the major organs towards the skin, before expulsion, whereas, fresh blood replenish the affected area (Ibn-ul-Kuf, 1935).

2 MATERIALS AND METHODS

The study was conducted during November 2012 to December 2014 at Eastern Specialist Clinic (PECHS), Karachi jointly by Brookes Health and Education Foundation and Faculty of Eastern Medicine, Hamdard University, Karachi.

2.1. Requirements and Equipments

Cupping chair (self-fabricated), transparent (polyvinyl) bell shaped small (30 ml) or larger

(60 ml) vacuum cupping cups of circumference of 1 to 3 inches (25 mm-75 mm), vacuum pump with pistol grip (U.S Global, Karachi), sterilized and disposal items included: gloves, surgical blade, cotton and medical gauze and micro-pore surgical tape (Medics shop at medicine market, Karachi), pyodine (Brookes Pharma, Pakistan) and honey (Al-Shifa) were purchased from the local market.

2.2. Inclusion Criteria

The patients (65), both genders aged between 20-65 years were enrolled in the trial after obtaining their informed consent.

Temperamental Evaluation:

A questionnaire has been designed to assess the temperament of the patients for analysis of the effect of cupping therapy on temperament.

2.3. Exclusion Criteria

Patients suffering from chronic serious illnesses, dehydration, diarrhea, hypertension, severe vomiting and uncontrolled diabetes, females during menstruation and pregnant women were excluded. Cupping was also avoided when the patient experiencing bleeding disorders, inflammation, skin infection, ulcer, or who are prone to bruising.

2.4. Instructions for the Patients

Prior to the cupping treatment regimen all the patients were briefed as follow:

- i) To be mentally prepared and willing to adopt this therapy without any fear.
- ii) They should undergo 4h of fasting before therapy.
- iii) It is an additional benefit, if the timings of the sessions are kept in mind such as weather conditions specially hot weather

evenings are preferable as the humors are in diluted form and facilitates the expulsion of toxin-rich blood.

- iv) Patient will undergo full investigation related to the disease especially diabetics. The requirement of biochemical assays and pathological data.
- v) Physical examination included recording of body temperature, pulse rate, respiratory rate and blood pressure.
- vi) Patient should take bath 4h prior of cupping therapy which is most favorable for superficial cuts for the therapy, however, in case of immediate bath deep cuts are recommended.
- vii) Abstain from consuming dairy products on the day of treatment.
- viii. After procedure food should not consumed for at least 1-2 h.
- ix) Avoid sleeping after treatment for a period of 1h.
- x) After strenuous exercise or coitus it is also prohibited.
- xi) No blood thinning medicines to be consumed before cupping therapy.

2.5. Cupping Procedure

The patients (n = 65) were diagnosed on the basis of their clinical history and temperamental evaluation into: Knee joint pain/arthritis (n = 16), Shoulder pain/muscular spasm/cervical pain (n = 17), Sciatica (n = 3), Varicose vein (n = 3), Stress/Anxiety/Depression (n = 10), Migraine (n = 6), Chronic constipation (n = 5) and Dyspepsia (n = 5).

They were made to sit comfortably on a cupping chair (Fig. 1) in a separate room and had to undergo 20-25 minutes treatment conducted in 5 phases as elaborated below:

Primary sucking: The cup of appropriate size (Fig. 2) was placed on the selected site which clung to the skin and was left for a

period of 3-5 mins. The positioning of the cup at the site of blood collection followed the method (Xue, O'Brien, 2003 and Tait, Brooks, Harstall, 2002) which is a combination of cupping and acupuncture model emphasizing that stimulation of specific points on the skin should be in complete harmony with the control systems of the body.



Fig. 1: Cupping chair

The cupping chair was specially fabricated and the patient was seated 1 minute prior to cupping procedure. The various parts of chair represented as: a) Head, b) Arms, c) Thorax, d) Buttocks, e) Knees, f) Lower limb and g) Foot.

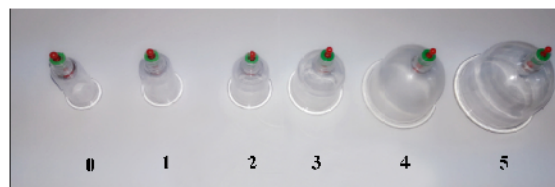


Fig. 2: Cupping cups of various sizes used for the collection of blood

The cupping cups of various size ranging from 0-5.

Scarification: Using surgical blades (gauge 15-22) superficial incisions 3-4 mm long and less than 0.1 mm deep were made on the skin.

- i) **Blood letting:** Immediately, the cup was placed on the skin, as described above for blood collection (3-5 mins) from the capillary vessels.
- ii) **Removal of cup:** Once the procedure was completed it was removed and antiseptic agent (Al-Shifa honey) was applied on the affected area.
- iii) **Dressing of affected area:** A pre-prepared medicated sterile fibrous material or sterile muslin or cotton wool was placed on it. This temporary dressing detached itself within an hour.

The cupping therapy consisted for 3-7 sessions (duration: 1½ month-3½ months) with an interval of 15 days. The cups of 4 and 5 size numbers (Al-Rubaye, 2012) were used throughout the procedure, while the position of cup on the front (thorax) and back (trunk) of the body was decided as described earlier (Cao, Han, Li *et al.*, 2010) with few modification and were either common or different depending on diseased conditions (Fig 3a and 3b).

3. RESULTS AND DISCUSSION

Patients (n = 65) underwent wet cupping therapy against 8 different ailments: 1) Constipation, 2) Dyspepsia, 3) Knee joint pain/ arthritis, 4) Migraine, 5) Shoulder pain/muscular spasm/cervical pain, 6) Sciatica, 7) Stress/ anxiety/depression and 8) Varicose vein and the results are presented in Table 1. The details of eight diseases are described individually depending upon the their level of response.

Constipation is generally defined as an inadequate defecation characterized by

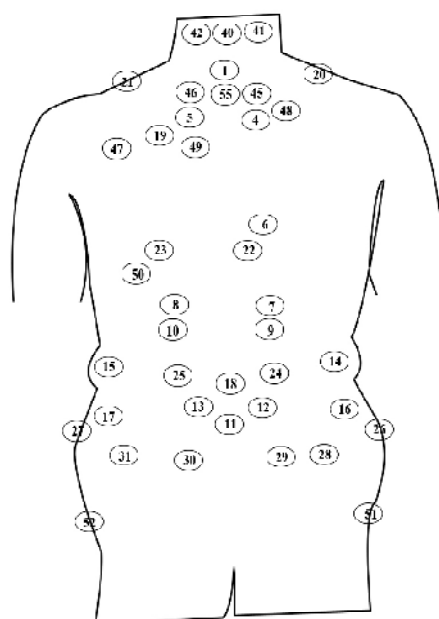


Fig. 3a: Cupping points on the front side of the human body

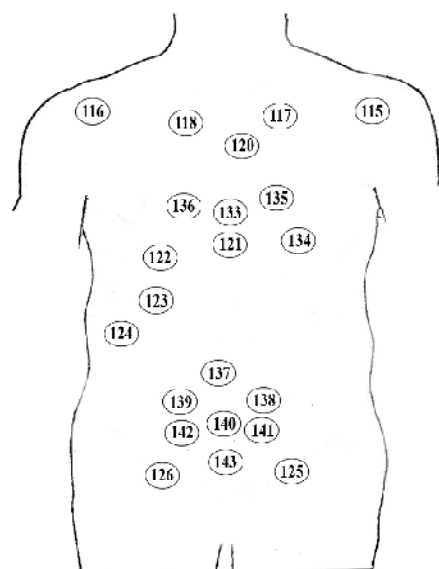


Fig. 3b: Cupping points on the back side of the human body

Various parts of body mostly used for cupping therapy. The numbers within circles indicate specific locations to place cups (5-7) as per disease condition.

Table 1: Responses of Cupping Therapy Against Different Disease Conditions in the Patients

Complaints and Respective parameters	n	Severity of Disease Ratings in Patients	Cupping Points	Response to Cupping Y or N (%) and Indications	Magnitude of Improvement and Percentage with Temperament
Chronic Constipation (Abdominal pain, hard or scanty stool. trouble having a bowel movement/straining, swollen tender abdomen, vomiting)	5	Mild = 2 Moderate = 3 Severe = 0	1, 55, 121, 6, 7, 8 137, 138, 139, 140, 141, 142 28, 29, 30, 31, 11, 12, 13	Y = 90 N = 0 Bowel movements normalized with improvement in digestion.	Mild = 0 Moderate = 10 (Melancholic) Marked = 90 (Sanguineous)
Sciatica (Radiating pain from lumbar region to the outer side of leg up till foot, difficulty in walking without support)	3	Mild = 0 Moderate = 2 Severe = 1	1, 55, 11, 12, 14, 16, 24, 26, 51, 132 1, 55, 11, 13, 15, 17, 25, 27, 52, 130	Y = 100 N = 0 Flexion and extension with ease	Mild = 0 Moderate = 10 (Melancholic) Marked = 90 (Phlegmatic)
Migraine (Blurred vision, headache-pulsating or throbbing pain on one or both sides of head, irritability, nausea and vomiting, sensitivity to light, sounds and sometimes smells)	6	Mild = 3 Moderate = 2 Severe = 1	1, 55, 20, 21, 23, 41, 42, 45, 46, 47, 48	Y = 90 N = 0 Pain episodes and interval prolonged up till a year	Mild = 0 Moderate = 10 (Bilious) Marked = 90 (Sanguineous)
Knee Joint pain/arthritis (Pain, reduced mobility, stiffness, swelling, tingling and numbness)	16	Mild = 5 Moderate = 9 Severe = 2	1, 46, 48, 7, 8, 11, 25, 24	Y = 80 N = 20 Movement with ease	Mild = 10 (Melancholic) Moderate = 30 (Bilious) Marked = 60 (Phlegmatic)

Complaints and Respective parameters	n	Severity of Disease Ratings in Patients	Cupping Points	Response to Cupping Y or N (%) and Indications	Magnitude of Improvement and Percentage with Temperament
Shoulder pain/muscular spasm/cervical pain (Muscle spasm, neck pain, and neck stiffness)	17	Mild = 3 Moderate = 13 Severe = 1	1, 55, 40, 41, 42, 11, 12, 13 49, 120, 4, 5, 6, 19	Y = 90 N = 10 Decreased pain, movement improved	Mild = 5 (Bilious) Moderate = 5 (Phlegmatic) Marked = 90 (Sanguineous)
Varicose vein (Enlarged and twisted veins, painful, strain during walking or long standing posture)	3	Mild = 03 Moderate = 3 Severe = 0	1, 55, 28,29, 30, 31, 132 127, 128, 12, 26, 27 51, 52	Y = 90 N = 10 Able to stand for longer durations	Mild = 0 Moderate =10 (Bilious) Marked = 90 (Sanguineous)
Stress/Anxiety/Depression (Irritability, mood swing, tension headaches, nausea, negative thinking)	10	Mild = 3 Moderate = 5 Severe = 2	1, 55, 11, 12, 13, 12 16, 46, 7, 8, 9, 10	Y = 70 N = 30 Mental stress lessened	Mild = 10 (Melancholic) Moderate =20 (Bilious) Marked = 70 (Phlegmatic)
Gastrointestinal disturbance (Abdominal pain, abdominal bloating, abdominal distension, belching, heart burn, loss of appetite, nausea, regurgitation, vomiting)	5	Mild = 1 Moderate = 3 Severe = 1	1, 55, 121, 6, 7 137, 138, 139, 140	Y = 90 N = 10 Symptoms relieved with improvement in digestive system.	Mild = 0 Moderate = 10 (Phlegmatic) Marked = 80 (Bilious)

Number of patients = n

Depending upon respective parameters for each disease within parenthesis, the rating scale for their severity is represented by a scale ranging from (0-10) and classified into:

Mild = (1-3), Moderate (4-6) and Severe = (7-10).

Cupping points: The numbers indicate the trigger points on the front and back of human body for corresponding disease (8)

Response to cupping therapy: Yes (Y) or No (N). The numbers indicate the percentage of patients responded to the therapy.

Magnitude of improvement in patients upon clinical observation of the symptoms is classified arbitrarily as: Mild, Moderate and Marked.

Temperament is shown within parenthesis [Sanguineous temperament *harr* (hot) and *ratb* (moist), phlegmatic temperament *barid* (cold) and *ratb* (moist), bilious temperament *harr* (hot) and *yabis* (dry), melancholic temperament, *barid* (cold) and *yabis* (dry)].

infrequent stools, difficult stool passage or both. It is well established that the pathophysiology of constipation under primary conditions are due to intrinsic problems of colon or anorectal functions, whereas, secondary causes are related to either organic (duodenal/jejunal perforations) and systemic diseases (hormonal, glandular) or to medications (antibiotic) (Brandt *et al.*, 2005, Higgins and Johanson, 2004 and Pare *et al.*, 2001). The patients suffering from constipation related to primary conditions, after 6 sessions of wet cupping therapy showed marked (90%) and moderate (10%) improvements as reflected by their normal bowel movements.

Sciatica is referred to a radiating pain or radicular pain arising from the hip joints radiating towards the outer side of the leg and foot with the features of numbness and weakness. The major cause refers to nerve compression resulting from severe arthritis, disc herniation and spinal stenosis. Sciatica-like-distribution called as pseudo-sciatica is resulted by the trigger points in the gluteal minimus muscle hence creating referred pain similar to radicular pain from sciatica. Trigger points are usually muscle "knots" tender to touch and if active result in pain that refers to other regions. It can be caused by abrupt or frequent burden, extended immobility, dislocation of the sacroiliac joint and nerve-root irritation. Trigger points (localized areas of muscle spasm) are targeted until the muscle tension subsides. For sciatic nerve pain, the trigger points that are often responsible for the nerve irritation are located in the gluteus medius and minimus muscles, as well as the gluteus maximus, piriformis, and the lumbar paraspinal muscles. (Vroomen *et al.*, 1999). In the present study, a scale of 0-10 (with 0 being no pain and 10 being the most painful) was used to locate the trigger points for sciatica. Patients (n = 3) suffering from

sciatica from 3-12 months complaint of tolerable pain with minimal disability, while patients experiencing intense leg pain with high disability scores and prolonged suffering were excluded. After wet cupping therapy for 5-8 sessions the flexion and extension of the legs particularly during walking showed marked improvement (90%) along with the reduction in inflammation of the lumbar region and enabled them to walk without support.

Migraine is a disturbance of sub-cortical aminergic sensory modulatory systems in the brain, or in brainstem, hypothalamic and thalamic structures. The cortical spreading depression (CSD) is associated with the activation of trigeminal nerve afferents which induces a series of brainstem and meningeal events appearing consistently at the time of migraine episode. Prompt CSD enhancement leads to a long-lasting increase in blood flow within the middle meningeal artery which is linked with the activation of trigeminal and parasympathetic system. (Stovner *et al.*, 2006). The patients in the study complaint of at least 4 hours of migraine pain or a day with which subsided after intake of pain killers (panadol, aspirin). The intensity headache scale ranged from 0 (no headache) to 10 (very severe headache). Efficacy of the treatment as a subjective evaluation by each patient was recorded at the end of the study as marked, moderate or mild improvement. Pain intensity and duration was also compared before and after treatment. In 6 patients after 3-10 sessions of wet cupping therapy a decrease in periodic pains and its duration was prolonged for about a year along indicating marked (90%) while and moderate (10%) improvement.

Knee Joint pain/arthritis can be caused due to cartilage destruction and the deposition of crystals in the joints from osteoarthritis (Levine *et al.*, 1986). Primary sensory (afferent) fibers linking peripheral receptors with second

order neurons in the spinal cord result in pain sensations arising from inflamed or damaged joints. Afferent fibers are capable of enhancing and diminishing their capacity to detect and respond to various stimuli. Inflammatory pain is associated with sensitization of sensory proteins at the nociceptive endings whereas pain that results from damage to the nerve or neuropathic pain has been linked to changes in axonal ion channels giving out ectopic discharge in nociceptors.

The patients complained about reduced mobility of knee, warmth or swelling, pain (stabbing, throbbing, burning, dull or sharp). Its intensity varied from mild to severe or tenderness, stiffness, tingling or other unusual sensations and numbness. It is well established that the joint pain mostly arises due to inflammation, cartilage degeneration, crystal deposition, infection, and trauma (Fauci *et al.*, 1998).

After treatment patients (n = 16) were relieved from pain, reflected as ease in joint movement and reduction in its stiffness. The improvement was evident in the patients after treatment shown within parenthesis and qualitatively classified as marked (60%), moderate (30%) and mild (10%). The patients displaying moderate and mild improvement could be due to unexplained fever, significant joint-movement disability accompanied by history of medication-induced immune-suppression as well use of steroidal injections prior to treatment.

Shoulder pain/muscular spasm/cervical pain is frequently result from compression or damage to the cervical spine nerve root reflected as pain along with the motor dysfunction, sensory deficits or adaptation in tendon reflexes. Cervical disc herniation and degenerative changes are its most common causes. Radiculopathy usually affects C5 to C7 levels

and weakness in sensory symptoms (Eddy and Loud, 2005).

Patient (n = 17) complaint about non-specific neck pain radiating into shoulder, upper back, arm(s) and head leading to neck stiffness and muscle spasm. Few patients also complained about unilateral neck, shoulder, or arm pain closer to dermatome. After 5-6 sessions of wet cupping therapy marked (90%), moderate (5%) and mild (5%) improvement was observed. It was noted that the symptoms were aggravated by particular movements, posture and other physical activities in patients showing mild or moderate improvement who were also suffering from cervical disc herniation and degenerative changes.

Varicose veins are enlarged and twisted most commonly observed on the legs. To prevent blood from flowing backwards that is retrograde flow or venous reflux, veins have pairs of leaflet valves leading to formation of cluster of veins that induces inflammation and pain (Mark, 2011).

3 patients were treated through wet cupping therapy applying 5-9 sessions. Results included easy movement, no inflammatory conditions, patient can walk with ease, there was marked improvement (90%) in the 2 patients and moderate (10%) in 1 patient was seen because of severity of the case. The majority of cases of varicose veins are considered to be benign although rigorous varicosities can lead to major complications due to the insufficient circulation of the affected limb.

Stress/Anxiety/Depression, 10 patients after 5-6 sessions of wet cupping therapy resulted in lessened mental stress indicating marked improvement (70%) in 6, moderate improvement (20%) in 3 and mild improvement (10%) was seen in 1 patient. The terms stress or depression are linked with neuronal atrophy characterized by loss of synaptic connections in

cortical and limbic brain regions implicated in depression. The concept considered to occur through decreased expression and function of growth factors like brain-derived neurotrophic factor in the prefrontal cortex and hippocampus. It's difficult for typical antidepressants to reverse structural alterations. According to the *Diagnostic and Statistical Manual of Mental Disorders*, 5th Edition (DSM-5), anxiety disorders include disorders that share features of excessive fear and anxiety and related behavioral disturbances.

Gastrointestinal disturbance including dyspepsia, vomiting, hyperacidity, 5 patients after 5 sessions of wet cupping therapy resulted in improvement of symptoms and better appetite indicating marked improvement (90%) in 4 patients and 1 had shown moderate improvement (70%). Dyspepsia is related to the symptoms such as nausea, vomiting and postprandial fullness. Symptoms such as postprandial pain, belching, and weight loss are associated with hypersensitivity to gastric distension. Psychosocial factors and altered response to duodenal lipids or acid have also been identified as pathophysiologic mechanisms.

The present study supports the earlier concept behind cupping therapy and the improvement in about 72% of patients observed could be related to the transferring of discomfort through evacuation of morbid matters from the affected area referred to as *Amalaa* or *Tanqiya-e-Mavad* leading to required blood circulation to the affected area thereby providing appropriate nutrients.

The marked improvement was linked with the temperament of the patients such as sanguineous, phlegmatic, bilious, melancholic and the observations were concluded as > sanguineous temperament *harr* (hot) and *ratb* (moist) > phlegmatic temperament *barid* (cold) and *ratb* (moist) > bilious temperament *harr*

(hot) and *yabis* (dry) > melancholic temperament, *barid* (cold) and *yabis* (dry). The therapeutic effect of wet cupping therapy followed a sequence of: chronic constipation > migraine > sciatica > shoulder/cervical/muscular pain > varicose veins > dyspepsia > knee joints pain > stress/anxiety/depression.

It has been noticed that in case of temperamental imbalance in the cold and dry qualities, there was an increased stiffness that aggravates the natural cold and dry qualities of the connective tissues, however, other associated factors like overweight, hormonal variations, excessive intake of cold and dry foods. Additionally, six essential factors of Unani medicine also play an important role in progression towards temperamental imbalance. In patients having sanguineous and phlegmatic temperament a marked improvement in their ill-health conditions, improved sleeping pattern as well as their emotional status.

The results presented above implies that in cupping therapy a correlation between temperament and improvement among patients exists, however further studies on larger scale are required to confirm our findings. Nevertheless, patients showing no response to cupping were either affected by the prolonged medication or severity of the disease indicated marked disability or any other organic disorder.

In some patients temporary skin discoloration was apparent that lasted for 3-5 days. Although, minor and temporary bruising in some patients were also prominent which were reversible but it needs to be taken into account for those who are sensitive to bruising. Nonetheless, most of patients were satisfied with the cupping therapy and its outcome because of its efficacy, safety and cost effectiveness. Considering the popularity of cupping in Pakistan and to minimize its misuse guidelines for its safety needs to be prepared

and implemented. Moreover, in order to achieve high level of medicinal significance along with complete safety and efficacy proper improvement in high techniques, scientifically designed instrumentation and advanced or ultra laboratory facilities for the selection of précised patients should also be needed to avail great benefits of cupping therapy.

It is concluded that if precautionary measures are taken, wet cupping therapy is effective against diseases of various origins and appeared to be depended on the severity of the disease and temperament of the patients. However, further studies are still required for other diseases as well as to prove or disapprove claims of health benefits.

Author's Contribution and Declaration

Tasneem Qureshi: Conducted the therapy.

Prof. Dr. Hakim Abdul Hannan: Supervised the research study.

The author's have no conflict of interest.

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***Cuscuta reflexa* L. & Roxb.: An Implication of Quercetin Mediated Monoamine Oxidase Inhibition in Preclinical Models of Depression**

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Abstract

The current study was aimed at exploring the folkloric use of *Cuscuta reflexa* dodder a parasitic plant (family convululaceae) against depression. Briefly, the methanolic extract of *C. reflexa* vine was assessed for antidepressant action using various behavioral paradigms such as tail suspension test (TST), forced swim test (FST) and locomotor activity test. The serotonergic and noradrenergic changes were evaluated using 5-hydroxytryptophan (5-HTP) induced head twitches and yohimbine potentiation tests, respectively. The fluoxetine and phenelzine were used as positive controls in the study. Our data demonstrated that the *C. reflexa* extract significantly declined the immobility time in TST ($EC_{50} \sim 50$ mg/kg) and FST without affecting the locomotor counts. The extract also significantly increased the 5-HTP induced head twitches and yohimbine induced lethality. The aforesaid behavioral outcomes were similar to that caused by standard drug phenelzine (monoamine oxidase inhibitor, MAOI). It is known that quercetin is an inhibitor of MAO and also the constituent of *C. reflexa*. Our HPLC data confirmed the presence of quercetin (0.156 mg/25 mg of extract; retention time = 2.1 minutes) in the extract and was used in present study. In conclusion, the present study validate the folkloric use of *C. reflexa* as an

antidepressant and this effect can be attributed to quercetin mediated rise in neuronal serotonin and noradrenaline levels possibly *via* MAO inhibition.

Keywords

Cuscuta reflexa L. & Roxb.; antidepressant; phenelzine; serotonin; noradrenaline.

1. INTRODUCTION

Depression is a complex mental disorder that presents itself as sadness, fatigue, worthlessness, disturbed weight/sleep, and loss of social interactions. According to World Health Organization (WHO) estimation, around 20% of the global population is affected from depression. Furthermore, it is the 3rd foremost cause of disability and would most likely be the first by 2030 (*The Global Burden of Disease*, WHO, 2004).

The complexity of depression can be reflected by numerous hypotheses that have been proposed underlying its pathogenesis. The most significant, among all, is the monoamine hypothesis which states that depression can be attributed to low levels of monoamine neurotransmitters (serotonin and noradrenaline) in the brain. The significance of this hypothesis can be reflected by the fact that most of the

clinically used antidepressants (SSRIs) possess monoamine elevation as core mechanism of action (Elhwuegi, 2004).

The treatment with medicinal plants is very common practice around the globe. These have been used as medicine since antiquity and many commercially used drugs are initially obtained from plants. The WHO estimates that approximately 75-80% of the world population (mainly in developing countries) uses medicinal plants against several diseases (Cass, 2004). *Cuscuta reflexa* (English name: Giant dodder) is a parasitic plant that grows widely in Pakistan. It belongs to the family Convolvulaceae and genus *cuscuta* comprising ~170 species. It is usually a very long, branched, twining vine that is found on several plants species. It fully or partially covers the host plant by its twining stem. It usually lacks chlorophyll or has meager amounts thus unable to undergo photosynthesis to synthesize its own food. It is a bitter plant with pungent odor. Our survey also revealed that it is traditionally used by 'hakims' for the treatment of depression and also reported to possess anxiolytic potential (Pal *et al.*, 2003). Keeping this in mind, the current study was designed to assess the antidepressant potential of *C. reflexa* using various preclinical behavioral paradigms.

2. MATERIALS AND METHODS

2.1. Animals

Sprague Dawley rats (150-250 g) and Balb/c mice (25-30 g) were obtained from the animal house facility of COMSATS Institute of Information Technology (CIIT), Abbottabad. The animals were placed in plastic cages, each cage comprised of ten animals at $25 \pm 1^\circ\text{C}$ and light/dark cycle of 12 h each. Food and water was freely and sufficiently available to all the animals. All experiments were performed according to the protocols of Animal Use and

Care Committee of the CIIT that is in accordance with the guidelines by National Institute of Health (NIH publication no. 85-23, revised 1985) for the care and use of laboratory animals.

2.2. Chemicals

The following chemicals are used: 5-Hydroxytryptophan supplement (100 mg capsules, Natrol, USA), ethanol (BDH laboratory, England), fluoxetine phenelzine and yohimbine (Sigma Aldrich, USA). Distilled water was used during entire course of experiments.

2.3. Preparation of Extract and Standardization

Cuscuta reflexa L. & Roxb (Fig. 1) parasite on *Ficus religiosa* L. was identified (Mr. Sher Wali, Herbarium Department, University of Karachi) and assigned a specimen voucher No. 44269. It was chopped (1 Kg) into small pieces and repeated (4 \times) extraction was performed with methanol (70%) at room temperature. The combined extract was later concentrated under vacuum affording thick syrup which constituted the crude methanolic



Fig. 1: *Cuscuta reflexa* L. & Roxb.
Host Plant: *Ficus religiosa*

extract. The extract was standardized against quercetin using HPLC-DAD (Agilent 1200, USA). Briefly, the methanolic extract of *C. reflexa* (25 mg/10ml) and quercetin (1 mg/5 ml) were injected (5 μ l) into HPLC containing C18 column (4.6 \times 50 mm, 1.8 μ , Zorbax SB, Agilent Technologies, USA) set at 20°C. The mobile phase (0.25% acetic acid, 72% water and 28% acetonitrile) was used at flow rate of 0.5 mL/min.

2.4. Tail Suspension Test (TST)

It was performed as described by Steru (Steru *et al.*, 1985). The animals were distributed in four groups (n=5) into: *C. reflexa* extract i) (25, 50 and 75 mg/kg), ii) fluoxetine (30 mg/kg), iii) phenelzine (30 mg/kg) and iv) vehicle (10 ml/kg). After 1 hr of intraperitoneal treatment, the animals were suspended by the tail (using adhesive tape) almost 15 cm above the ground for 6 minutes. The immobility time (when the animal was static) was videotaped for 5 minutes (excluding the first minutes) and compared with that of control.

2.5. Locomotors Activity Test

Prior to performing this test, the animals were placed in activity cage (1.5 by 1 ft. box having 2 \times 2 inch markings on the floor) for 5 minutes for acclimatization. Later, these mice were treated (intraperitoneal) with *C. reflexa* extract (IC₅₀ dose), phenelzine (30 mg/kg), fluoxetine (30 mg/kg) or vehicle (10 ml/kg). After 1 h, the animals were placed again in the activity cage for 6 minutes and the number of boxes crossed by animals were recorded for 5 minutes and compared with the control.

2.6. Forced Swim Test (FST)

FST was performed as detailed by Porsolt (Porsolt *et al.*, 1977). This test involved the use of a glass tank (17 \times 17 \times 50 cm) filled with

water (25 \pm 1°C) up to 20 cm. The test was performed in two sessions i.e. pre-test and test session with a time lapse of 24 hours. In the pre-test session (15 minutes) male rats were placed in the FST tank. The animals showing nose bleeding during pre-test session were excluded from the experiment. After 24 hours, the healthy animals was treated (intraperitoneal) with *C. reflexa* extract (IC₅₀ dose), fluoxetine (30 mg/kg), phenelzine (30 mg/kg) or vehicle (5 ml/kg). The treated animals after 1 hour were individually exposed for 6 minutes to FST session and immobility time (animal is motionless or making slight motions to keep the head above water) was recorded for last 5 minutes (providing 1st minute for familiarization) and compared with control.

2.7. 5-hydroxytryptophan Induced Head Twitches

Test animals (n=5/group) were treated with 5-HTP (100 mg/kg, *i.p.*). After 30 minutes, these animals were again treated with *C. reflexa* extract (IC₅₀ dose) or fluoxetine (30 mg/kg) and head twitches were counted for 30 minutes (Sallinen *et al.*, 1998).

2.8. Yohimbine Potentiation Test

In this test, mice were divided into three groups (n=5) i) Yohimbine (40mg/kg dissolved in ethanol) or in addition (30 minutes before yohimbine) with ii) *C. reflexa* (IC₅₀ dose) or iii) phenelzine (30 mg/kg). After 24 hours, percentage of lethality was calculated (Quinton, 2012).

2.9. Statistics

The data is presented as mean \pm SEM (n = 5-10 / dose). Differences between various means were computed by using one-way ANOVA followed by least significant difference (LSD). The level of significance is: *p<0.05,

p<0.01 and *p<0.005 as compared to their respective control.

3. RESULTS AND DISCUSSION

The present investigation was aimed to explore the traditional use of *C. reflexa* in depression. The tail suspension test has been popularly employed for assessment of antidepressant activity (Steru *et al.*, 1985). In similarity with standard antidepressants (phenelzine and fluoxetine), the extract significantly reduced the immobility time (EC_{50} dose = 50 mg/kg) thereby supporting its traditional use (Table 1). As per literature, the psychomotor stimulants may lead to false positive results in test (Cryan *et al.*, 2002). Therefore, the locomotor activity test was also performed in order to confirm the antidepressant action and rule out any possibility of false results

caused by stimulants. Our results clearly showed that *C. reflexa* did not significantly increase the locomotor activity (data not shown) thereby certifying its antidepressant property. The literature also supported its other neurological effects as it is shown to be neuro-protective (Aruoma *et al.*, 2003), anxiolytic (Pal *et al.*, 2003) and anticonvulsant (Mehrabani *et al.*, 2007) in nature.

Another widely used tool to assess the antidepressant activity is FST. Its major drawback is failure to detect SSRIs i.e. selective serotonin reuptake inhibitors (Cryan *et al.*, 2002). Likewise, fluoxetine failed to manifest its action, while phenelzine (monoamine oxidase inhibitor, MAOI) reduced the immobility time (Table 1). Interestingly, the *C. reflexa* extract also displayed activity similar to phenelzine. Keeping in view the results, it can be deduced

Table 1: Effect of *C. reflexa* Extract on the Immobility Time of Rodents in Tail Suspension Test and Forced Swim Test

Treatment	TST		FST	
	Dose (mg/kg)	Mean immobility time	Dose (mg/kg)	Mean immobility time
<i>C. reflexa</i> extract	25	144±15	50	102±13***
	50	82±6***		
	75	131±9**		
Phenelzine	30	90±11***	30	139±8*
Fluoxetine	30	56±8***	30	201±11

The values represent the mean immobility time ± S.E.M (n = 5).

Mean immobility time of control animals: 155±7 (TST) and 189±12 seconds (FST).

Asterisk(s) indicate the significance levels i.e. *p<0.05, **p<0.01 and ***p<0.005 as compared to respective control.

that the extract probably belongs to the MAOI class of antidepressants but more studies in presence of MAOI inhibitors are required for its verification.

The extract of *C. reflexa* was further subjected to different behavioural tests (at EC₅₀ dose i.e. 50 mg/kg) in order to incriminate the monoaminergic modulation. The 5-hydroxytryptophan (5-HTP) is an amino acid that acts as precursor of monoamine neurotransmitter serotonin, whose high level manifests serotonin syndrome characterized by symptoms such as tremors. The 5-HTP triggered head twitch test is used to identify the effect of antidepressant on serotonergic function (Sallinen *et al.*, 1998). Our data revealed that in conformity with fluoxetine, the head twitches triggered by 5-HTP were significantly ($p < 0.005$) enhanced by *C. reflexa* (50 mg/kg) (Table 2) suggesting that it is probably acting *via* elevating the levels of serotonin in the brain.

Yohimbine is α_2 -antagonist and its

potentiating test has been frequently used in order to signify the effect of substances that cause noradrenaline modulation (Quinton, 2012). Our study demonstrated that the lethality caused by yohimbine (35%) alone was raised to 70 % and 90 % after treatment with either *C. reflexa* (50 mg/kg) or phenelzine (30 mg/kg; Table 2), respectively. This led us to suggest that *C. reflexa* most likely has the ability to elevate levels of noradrenaline at neuronal level.

The aforementioned behavioural effects caused by *C. reflexa* extract are similar to those caused by standard drug phenelzine that is a monoamine oxidase inhibitor (MAOI). In search of MAOI in extract, it was found through literature that the main active constituents in *C. reflexa* are amebel, quercetin, cuscutine and cuscutamide (Patel *et al.*, 2012). Among all, the quercetin was reported to possess the monoamine oxidase inhibitory potential (Singh *et al.*, 2003; Yoshino *et al.*, 2011). Our HPLC data confirmed that the extract contains

Table 2: Effect of *C. reflexa* Extract on 5-HTP Induced Head Twitches and Yohimbine Lethality Test

5-HTP induced head twitches			Yohimbine induced lethality		
Treatment	Dose (mg/kg)	Number of head twitches	Treatment	Dose (mg/kg)	Mortality (%)
5-HTP	200	29 ± 5	Yohimbine	40	35
Fluoxetine + 5-HTP	30+200	100 ± 11***	Phenelzine+ Yohimbine	30+40	90
<i>C. reflexa</i> + 5-HTP	50+200	79 ± 6***	<i>C. reflexa</i> + Yohimbine	50+40	70

The values represent the mean number of head twitches ± S.E.M (5-HTP induced head twitches) and percent mortality in mice (yohimbine induced lethality) (n = 10).

Asterisk(s) indicate the significance levels *** $p < 0.005$ as compared to respective control.

0.156 mg of quercetin (retention time = 2.1 minutes) per 25 mg of methanolic extract of *C. reflexa* (Fig. 2).

In conclusion, the *C. reflexa* extract elicited antidepressant effect that justifies its folkloric use. This action can be attributed to quercetin mediated increase of serotonin and noradrenaline levels in the brain probably *via* monoamine oxidase inhibition.

Author's Contribution and Declaration

Ms. Sara Zeeshan and Mr. Wahid Zada have performed the bench work.

Dr. Huma Aslam Bhatti has prepared the extract and standardized it against quercetin.

Dr. Ghulam Abbas has supervised the entire work.

The authors declare that there is no conflict of interest.

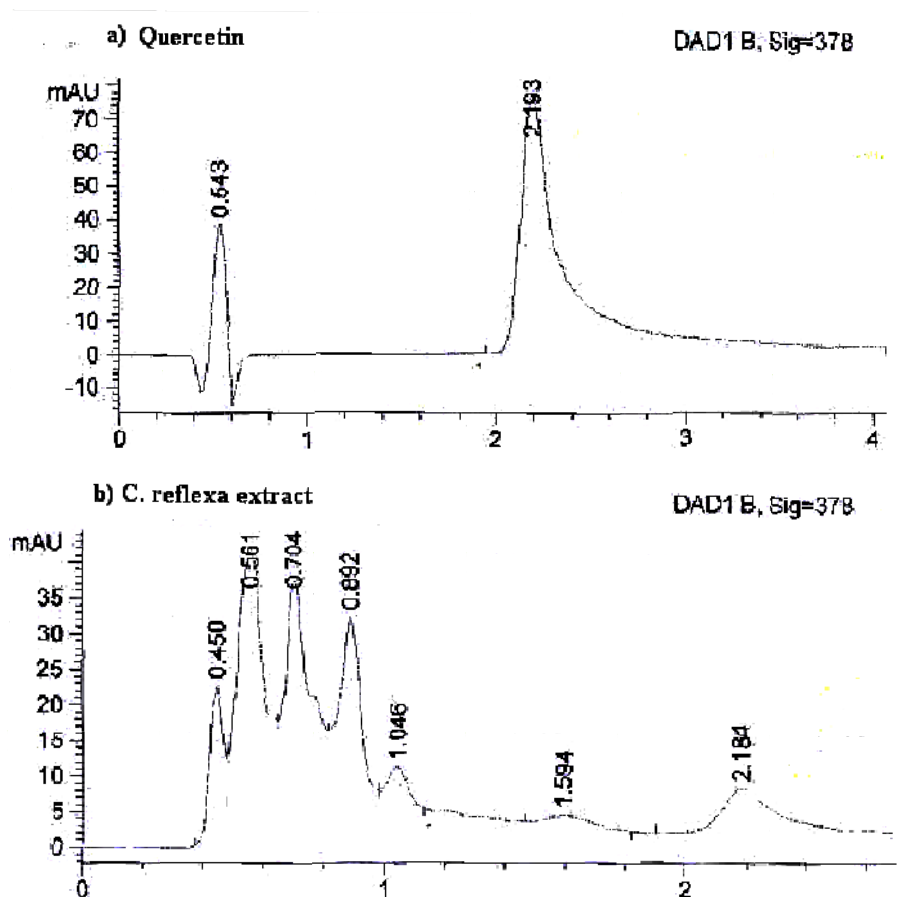


Fig. 2: Identification of quercetin in *Cuscuta reflexa* methanolic extract using HPLC-DAD
HPLC-DAD chromatograms of: (a) Quercetin (1 mg/5 ml, retention time = 2.1 min) and (b) *C. reflexa* extract (25 mg/10 ml) showing the quercetin peak at 2.1 min which is equivalent to 0.156 mg.

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Anti-bacterial Activity and Qualitative Phytochemical Screening of *Fagonia cretica* L.

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Abstract

Fagonia cretica L. is traditionally used against many diseases including gastrointestinal and respiratory ailments. The present study reports its minimal inhibitory concentration against bacterial strains. Thus crude methanolic extracts of *F. cretica* was evaluated against 17 bacterial pathogenic strains and effective only against 17% of bacterial cultures as reflected by *Proteus vulgaris*, *Micrococcus luteus* and *Staphylococcus saprophyticus* which showed minimal inhibitory concentration of 50 µg/ml, 50 µg/ml and 100 µg/ml, respectively with possibly due to synergistic actions of chemical composition but selectivity towards *M. luteus* and *Proteus vulgaris*. Further fractionation of *F. cretica* crude methanolic extracts with hexane, ethyl acetate and water enhanced bactericidal activity (MIC 25 µg/ml) of the respective plant fractions against *P. vulgaris*. Similar magnitude of response with hexane and ethyl acetate fractions against *Salmonella typhimurium* were also noted, whereas crude extract was ineffective. A qualitative phytochemical screening revealed that *F. cretica* methanolic extract coumarins and diterpenes predominates while in water fraction it is saponins and hence are possibly

be associated with antibacterial activity. On the other hand leucoanthocyanins and diterpenes were absent. Thus present study provides a scientific rational for medicinal use of *F. cretica* L. to treat gastrointestinal, urinary tract and respiratory tracts infections in humans. Further studies are required to isolate and identify the active coumarins, diterpenes and saponins-based lead molecules.

Keywords

Fagonia cretica; bactericidal activity; *S. saprophyticus*; *P. vulgaris*; *S. typhimurium*; *M. luteus*; qualitative phytochemical screening.

1. INTRODUCTION

Medicinal plants have been used traditionally for the treatments of various ailments since ancient time which laid the foundation of currently available phytochemicals with therapeutic properties and also component(s) of many synthetic drugs. Atropine, ephedrine, digoxin, morphine, quinine, aspirin and reserpine are few such examples of drugs, invented based on folk knowledge of indigenous people (Gilani and Atta-ur-Rahman, 2005). The importance of plants with curing properties has been realized by researcher as

their medicinal uses have been associated with ~95,000 wild plants out of which only a small fraction (~7,500 plants) are used as a source of medicine, 3,900 as edibles, 400 as a source of fodder, 500 as fibre producers and 300 being used as bio-pesticides (Mishra *et al.*, 2007). Despite of a large number of plants only ~700 plant species have been exploited as food, medicinal source, flavouring agents and by cosmetic industry etc (Shinwari, 2010). In developing countries including Pakistan, ~80% of the people rely on folk medicines using natural products from medicinally important plants for primary health-care (Igbinsosa *et al.*, 2009).

It is well established that human pathogenic bacterial strains with the passage of time have developed resistance against antibiotics accompanied by various undesirable side effects. Therefore, it is crucial to explore alternatives to combat bacterial infections and in this context natural products from other sources particularly medicinal plants with significant potential to provide drug candidate(s) because of their rich molecular diversity and likelihood of producing lesser side effects.

Fagonia cretica, bitter in taste is used for the treatment of cancer, fever, thirst, vomiting, dysentery asthma, urinary discharges, liver and kidney ailments, typhoid, toothache, stomach discomforts and skin diseases (Pareek *et al.*, 2012 and Gulshan *et al.*, 2012). It is an antioxidant, febrifuge, astringent, antimicrobial and prophylactic against small pox The powder of whole plant is dusted on boils and skin eruptions (Qureshi *et al.*, 2010) and decoction given orally is also effective for curing skin eruptions. Its herbal tea has been reported to be effective against breast cancer (Lam *et al.*, 2012). The leaves and twigs showed anti-venom potentials against *Naja naja karachiensis* (Razi *et al.*, 2011). *F. cretica* possesses

hepatoprotective (Qureshi *et al.*, 2016; Bagban *et al.*, 2012), antitumor, cytotoxic with dipeptidyl peptidase-4 (DPP-4) inhibitory properties (Hussain *et al.*, 2007; Saleem *et al.*, 2014).

The 7th largest desert in the world also known as the 'Great Indian Desert' is located in the Sindh province of Pakistan as well as in Rajasthan, India. It covers an area of about 2,00,000 sq. Kilometres and divided into three parts in Pakistan. The Northern part is in South end of Punjab it is called 'Cholistan', spreading over an area of 16,000 square kilometers (30 km from Bahawalpur city). The locals are nomadic herders living in huts made of mud and straw. Cholistan desert of Pakistan is a rich land bestowed with many medicinal plants. It is hypothesized that the harsh weather conditions of this desert (winters with low temperature touching the freezing point with frost formation, while the summers are hot and dry with temperature rising to 51°C) plays a pivotal role in inducing and modifying secondary metabolites in the plants possessing plethora of medicinal properties. Although, its antibacterial and antifungal potential were associated with flowers (Thetwar *et al.*, 2006; Vaibhav *et al.*, 2014)



Fig. 1: *Fagonia cretica* Auct-non Linn Parker

however, information regarding its minimal inhibitory concentration is lacking. Therefore, based on ethnomedicine and ethnobotanical information available for *F. cretica* from Cholistan Desert it was evaluated for antibacterial activities against pathogenic and non-pathogenic bacterial strains.

2. MATERIALS AND METHODS

2.1. Plant Material

Healthy *F. cretica* bearing leaves, stems, roots and flowers were collected from Chak Lehar a location of Cholistan desert. After identification by the taxonomist Mr. M. Warris a voucher number was assigned (3430/CIDS/IUB) and the specimen deposited in the herbarium.

2.2. Extraction

The plant was shade dried and milled to obtain its powder (6.0 kg) that was soaked in methanol (10 L) for a week, followed by filtration and evaporation under high vacuum. The extraction process was repeated twice with methanol (7.5 L) for 7 days, yielding 64 g of methanol extract.

The *F. cretica* crude methanolic extract (11 g) was dissolved in of distilled water (50 mL) and solvent-solvent successive extractions were performed with petroleum ether (50 mL × 3) to obtain hexane extracts (0.36 g). Thereafter, extraction of aqueous extracts with ethyl acetate layer (50 mL × 3) afforded ethyl acetate extracts (0.45 g).

2.3. Bacterial Cultures

The bacterial strains were generously provided by Microbiology Laboratory of Attaur-Rahman, School of Bio-Sciences (ASAB) Islamabad, Pakistan. The Gram-positive strains included: *Micrococcus luteus* ATCC-4617,

Staphylococcus aureus, *S. aureus* ATCC, *S. Saprophyticus*, and *Streptococcus pyogenes*. The Gram-negative strains comprised: *Acinobacter junii*, *Bordetella bronchiseptica*, *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Serratia marcescens*, *Shigella dysenteriae*, *Salmonella paratyphi*, and *S. typhimurium* ATCC-14097.

2.4. Chemicals

The following chemicals are used: Agar, organic solvents, isoamyl alcohol, sodium hydroxide and copper sulphate were purchased from Sigma (USA). All the other chemicals used were of analytical grade.

2.4. Antibacterial Screening

The sensitivity of different bacterial strains to *F. cretica* extracts was measured by popularly used agar diffusion assay (Bauer *et al.*, 1966). For bacterial culturing, Luria Bertani agar (28 gm) in distilled water (1 L) was autoclaved for 15 min at 121°C. The sterilized media was poured into petri dishes and left at room temperature for 20-30 minutes. Modified method of Kirby-Bauer was used for inoculation of bacterial cultures in petri plates using sterile streaking loop by streaking in three different angles keeping the same central position to acquire uniform bacterial colonies. Appropriate precautionary measures were also taken to avoid contamination with other cultures and the streaking-loop was sterilized on red hot flame, before using new bacterial culture. The organic extracts were screened for their antibacterial activity using a established disc diffusion method (Jamil *et al.*, 2012). A 6 mm filter paper discs were prepared, autoclaved and a 10 µl sample of organic extracts (100 mg/ml) were applied individually. While on control plate

three discs without any sample was placed. Two standard drugs gentamicin and ciprofloxacin served as positive controls and dimethylsulfoxide (DMSO, 50%) were used as a negative control. All the plates were incubated at 37°C overnight for about 18-20 hours and zone of inhibition (mm) were measured. The experiments were performed in triplicate.

2.5. Minimum Inhibitory Concentration (MIC)

In the disc diffusion assay, the average size of the zone of inhibition ($n = 3$) is presented for those extracts that showed a consistent and noticeable clear zone around the filter paper disc (6 mm). The inhibitory zones of crude methanolic extract, hexane, ethyl acetate and aqueous fractions were expressed as the mean \pm error and compared with control.

2.6. Phytochemical Screening

The phytochemicals such as coumarins, diterpenes, leucoanthocyanins, saponins and steroids were determined in methanolic extracts and aqueous fraction as described earlier (Brain and Turner, 1975).

Coumarins

Yellow colour was evident after addition of 3 mL NaOH (10%) in 2 mL of test substance.

Diterpenes

The extract and fraction were dissolved in water and upon addition of copper sulphate emerald green colour appeared, indicating the presence of diterpenes.

Leucoanthocyanins

Addition of isoamyl alcohol (1.0 mL) to test substances (1 mL) formed red colour in

the upper layers indicating presence of leucoanthocyanins.

Saponins

On the addition of distilled water (5 mL) to 10 mL of test substances followed by vigorous shaking caused stable foam that was considered as an indicative of saponins.

Steroids

Equal volume of concentrated sulphuric acid and chloroform (10 mL) introduced in the test substances. The appearance of red colour in the upper layer and yellowish green colour in the lower layer confirmed the presence of steroids.

3. RESULTS AND DISCUSSION

In different parts of Cholistan desert the local dwellers depends on infusions and decoctions of *F. cretica* that is prepared using water as a solvent for curing multiple ailments. Thus based on ethnomedicine and ethnobotanical information *F. cretica* was evaluated for their antibacterial actions against 17 bacterial species that vary with respect to their disease modality and virulence. For example, *E. Coli*, human enteric species is generally avirulent but sometimes causes weak virulent gastroenteritis and urinary tract infections and *S. typhimurium*, causing enterocolitis are both Gram-negative pathogens. While, *S. aureus* a Gram-positive pathogen is most common cause of abscess, food poisoning, and toxic shock syndrome; both are considered to be more virulent than *S. lactis* and *E. coli* (Levinson, 2008).

Our results demonstrated that *F. cretica* crude methnolic extract and its hexane, ethyl acetate and water fractions induced zones of inhibition against *P. vulgaris*. The crude methanolic extracts at 50 μ g and 100 μ g concentrations killed the bacteria in dose

Table 1a: Effect of *F. cretica* Methanol Extract and Hexane, Ethyl Acetate and Water Fractions on Gram Negative Bacterial Growth Using Disc Assay

Test agents	$\mu\text{g/ml}$	<i>A. juiti</i>	<i>B. bronchiseptica</i>	<i>E. coli</i>	<i>E. cloacae</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>P. vulgaris</i>	<i>S. typhi</i>	<i>S. marcescens</i>	<i>S. dysenteriae</i>	<i>S. paratyphi</i>	<i>S. typhimurium</i> ATCC-14079
Methanolic extract	25	-	-	-	-	-	-	-	-	-	-	-	-
	50	-	-	-	-	-	-	16.6 \pm 0.5	-	-	-	-	-
	100	-	-	-	-	-	-	24.8 \pm 0.3	-	-	-	-	-
n- hexane	10	-	-	-	-	-	-	-	-	-	-	-	-
	15	-	-	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	15 \pm 0.2	-	-	-	-	6.7 \pm 0.3
Ethyl acetate	10	-	-	-	-	-	-	-	-	-	-	-	-
	15	-	-	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	7.9 \pm 0.2	-	-	-	-	13.4 \pm 0.5
Water	10	-	-	-	-	-	-	-	-	-	-	-	-
	15	-	-	-	-	-	-	-	-	-	-	-	-
	25	-	-	-	-	-	-	13.5 \pm 0.5	-	-	-	-	-
DMSO		-	-	-	-	-	-	-	-	-	-	-	-
Gentamicin	10	7 \pm 0.2	23.4 \pm 0.4	7.1 \pm 0.1	-	7.0 \pm 0.1	-	12.3 \pm 0.6	-	7.8 \pm 0.3	10.7 \pm 0.5	-	20.1 \pm 0.2
Ciprofloxacin	10	-	34.9 \pm 0.2	6.9 \pm 0.1	21.8 \pm 0.4	5.8 \pm 0.3	11.9 \pm 0.1	29.6 \pm 0.5	24.8 \pm 0.2	24.9 \pm 0.4	31.1 \pm 0.2	21.4 \pm 0.5	30.1 \pm 0.1

The values represent mean \pm SEM as diameter (mm) zone of inhibition of 3 independent experiments
 Dimethylsulphoxide (DMSO, negative) and gentamicin and ciprofloxacin (positive) served as respective controls
 No effect (-).

dependent manner forming 16.6 mm and 24.8 mm of inhibitory zones, respectively. While the hexane fraction was more effective as a 15 mm zone was evident at lower concentration (25 µg). The ethyl acetate and water fractions at 25 µg also showed zone of inhibition of 7.9 mm and 13.5 mm, magnitude, respectively. Thus it can be inferred that chemical constituents of *F. cretica* act synergistically to inhibit the growth of *P. vulgaris* which is reputed for urinary tract and wound infections in humans. Interestingly, crude methanolic extracts of

F. cretica did not show any inhibition against *S. typhimurium* ATCC 14097 which is known to cause gastroenteritis and fever. However, at 25 µg both hexane and ethyl acetate fractions showed zones of inhibition of 6.7 mm and 13.4 mm (Table 1a) suggesting that bioassay guided fractionation will be the most suitable strategy to isolate active principles against the aforementioned diseases induced by *S. typhimurium*.

The crude methanolic extracts of *F. cretica* (100 µg) also induced

Table 1b: Effect of *F. cretica* Methanol Extract and Hexane, Ethyl Acetate and Water Fractions on Gram Positive Bacterial Growth Using Disc Assay

Test agents	µg/ml	<i>M. luteus</i> ATCC-4617	<i>S. aureus</i>	<i>S. aureus</i> ATCC	<i>S. pyogenes</i>	<i>S. saprophyticus</i>
Methanolic extract	25	–	–	–	–	–
	50	–	–	–	–	–
	100	6.7±0.3	–	–	–	10.7±0.2
n-hexane	10	–	–	–	–	–
	15	–	–	–	–	–
	25	–	–	–	–	–
Ethyl acetate	10	–	–	–	–	–
	15	–	–	–	–	–
	25	–	–	–	–	–
Water	10	–	–	–	–	–
	15	–	–	–	–	–
	25	–	–	–	–	–
DMSO		–	–	–	–	–
Gentamicin	10	23.5±0.5	34.1±0.1	26.5±0.5	12.7±0.2	14.6±0.5
Ciprofloxacin	10	30.3±0.5	23.2±0.4	39.6±0.5	30.9±0.1	29.9±0.1

The values represents mean ± SEM as diameter (mm) zone of inhibition of 3 independent experiments Dimethylsulphoxide (DMSO, negative), gentamicin and ciprofloxacin (positive) served as respective controls No effect (–).

S. saprophyticus growth inhibition (10.7 mm). Likewise, inhibitory zone of 6.7 mm at 50 µg against *M. Luteus* was also observed (Table 1b). However, all the fractions of *F. cretica* (such as hexane, ethyl acetate and water extracts) were ineffective for both bacterial cultures supporting synergism again in this particular case and also supporting the notion that combination of different chemical constituents together are more effective. The minimal inhibitory concentrations of the fractions against bacterial cultures is 2× lower than the corresponding extracts (Table 2) clearly supports that bioassay directed fractionation is required to see if the MIC values could be further improved.

This is to be noticed that crude methanolic extracts of *F. cretica* and its fractions were ineffective against 76% of bacterial isolates including both Gram-positive (*Staphylococcus aureus*, *S. aureus* ATCC, and *Streptococcus pyogenes*) and Gram-negative strains

(*Acinobacter junii*, *Bordetella bronchiseptica*, *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Serratia marcescens*, *Shigella dysenteriae*, *Salmonella paratyphi*), thereby suggesting that its selectivity towards *S. saprophyticus*; *P. vulgaris*; *S. typhimurium*; *M. luteus* emphasize to conduct in-depth studies on the extract and fractions in the diseases affiliated with them and also elucidate the mechanism of bactericidal activities residing in the plant.

The DMSO was used as a negative control as expected showed no effect on the bacterial cultures on the contrary, gentamicin and ciprofloxacin serving as positive controls were effective against all the strains used (Tables 1a and b) as reflected by observable zone of inhibition around bacterial colonies. Ciprofloxacin, fluorinated quinolones structurally related to nalidixic acid acts by inhibiting bacterial DNA gyrase. It is a broad spectrum antibacterial

Table 2: Minimum Inhibitory Concentration of *F. cretica* Methanolic Extract and Fractions Against Bacterial Strains

Bacterial strains	Extract/Fractions	µg/ml
<i>S. saprophyticus</i>	Methanolic Extract	100
<i>P. vulgaris</i>	Methanolic Extract	50
	Hexane Fraction	25
	Ethyl acetate Fraction	25
<i>S. typhimurium</i> ATCC 14079	Water Fraction	25
	Hexane Fraction	25
<i>M. luteus</i> ATCC 4617	Ethyl acetate Fraction	25
	Methanolic Extract	50

The MIC of ciprofloxacin and gentamicin = 10 µg/ml.

drug as most of Gram-negative bacteria are highly susceptible *in vitro* and many Gram-positive bacteria are moderately susceptible to it. Similar pattern was also observed in our study as only *Ajunii* was resistant to it. The other standard antibiotic used was gentamicin that acts by irreversibly binding to the 30S subunit of the bacterial ribosome, interrupting protein synthesis which is similar to other aminoglycosides. In the present study only *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella paratyphi* were resistant to gentamicin. Furthermore, small variations in the sensitivity of bacterial culture to plant extract and fractions and to standard drugs could also be attributed to the differences in growth rate of the tested organisms, nutritional requirements, temperature and inoculum size (Gaill and Jon, 1995) but all the precautionary measures were undertaken as described above. These results advocate that the methodology adopted for the anti-bacterial screening was accurate and supports that antimicrobial property residing in *F. cretica*

hence provides a appreciable source of alternate to antibiotics which are either not accessible and if so are not affordable. Considering that in rural areas herbal treatments are favored over the allopathic ones for their low cost and the rich traditional knowledge about healing systems as well as compatibility to the human physiological system. Our results favoured that crude extracts of *F. cretica* possess bactericidal activities. The results affirmed the claim by the herbal practitioners of the area and this plant can be used to treat gastrointestinal and respiratory tracts infections in humans.

The phytochemical analysis of the crude methanolic extracts and aqueous fraction of *F. cretica*, coumarins and diterpenes were predominant while, saponins and steroids were present in small amounts while leucoanthocyanins was absent. On the contrary, in the water fraction saponins was predominant followed by coumarins and steroids while diterpenes and leucoanthocyanins were absent (Table 3). Thus coumarins, diterpenes and saponins are possibly be associated with

Table 3: Qualitative Phytochemical Analysis of *F. cretica* Methanolic Extract and Aqueous Fraction

Chemical Groups	Methanolic Extract	Aqueous Fraction
Coumarins	+++	++
Diterpenes	+++	-
Leucoanthocyanins	-	-
Saponins	++	+++
Steroids	++	+

The presence of the chemical group is represented as:
Strong (+++), moderate (++), weak (+) and absent (-)

antibacterial activity as they were predominantly present in *F. cretica* methanolic extract and aqueous fraction, while leucoanthocyanins and diterpenes were absent.

It is concluded that, this study provides a scientific rationale for medicinal use of *F. cretica* against gastrointestinal and respiratory tracts infections in humans. However, further studies are required to explore its mechanism(s) of action of plants extracts and its secondary metabolite following bioassay directed approach with emphasis on coumarins and diterpenes and saponins. In parallel the synergistic effects noted also demands in-depth study in this direction along with standardization of plant that can also prove a worthy asset of Pakistani herbal medicines.

Author's Contribution and Declaration

Ms. Kashaf Zia, Ms. Saima Hanif, and Ms. Alia Sadiqa performed the bench work.

Dr. Muhammad Qasim Hayat and Prof. Dr. Shazia Anjum have supervised the entire work.

The authors declare that there is no conflict of interest.

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Unani Treatment of Tinea Capitis: A Case Study

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Abstract

Tinea capitis (TC) (سَعْفَةُ الرَّأْسِ) is a dermatophytic infection of the scalp this infection most commonly affect children usually under the age of 12 years and frequently seen in boys. This is a case study of an 11 year old boy naïve patient who presented to the OPD with a history of severe scaly, crusting, bald patches on scalp from 5months. The clinical diagnosis was confirmed to be TC and the causative organism is *Trichophyton violaceum*. After treatment with polyherbal formulation (Nuskha No. 9 and 10) for a period of 8-weeks with follow up the sign and symptoms of the patient resolved completely with healthy normal hair growth pattern. This case study confirmed that nuskha No. 9 and 10 are effective against TC and it is cost effective. However, more data is required to justify its efficacy, safety and its mechanism of action.

Keywords

Unani treatment, Tinea capitis, Case study.

1. INTRODUCTION

Tinea capitis (TC) represents tinea (fungal) and capitis (scalp) is a dermatophytic infection of the scalp (Elewski, 2000). The dermatophytes comprise of three genera: a) *Trichophyton*, b) *Epidermophyton* and c) *Microspora*. (Gupta and Summerbell, 2000). All of them grow in keratinized environment

such as hair, nail and skin. They are further divided into 3 major groups: Anthropophilic dermatophytes which are limited to the human host only causing only mild chronic inflammation. Zoophilic dermatophytes are transmitted from pets, livestock and horses to humans resulting in marked inflammatory reactions in the host. The last group is the geophilic and its source is soil and can infect both human and animals (Tan, 2005).

Dermatophytes invade and multiply within keratinized tissues and classified into three main groups: trichophyton (skin, hair and nails), epidermophyton (skin and nails) and microsporum (skin and hair). It is characterized by erythema, scaling, pruritus and alopecia and its transmission occurs via infected persons and animal vectors through shedding of hair. Depending upon the site of infection, these have been classified clinically into TC (head), tinea faciei (face), tinea barbae (beard), tinea corporis (body), tinea manus (hand), tinea cruris (groin), tinea pedis (foot), and tinea unguium (nail) (Bennassar and Grimalt, 2010). Many fungal species can cause TC such as *Microsporum canis* (common in Europe), *Epidermophyton floccosum*, *Trichophyton mentagrophytes*, *Trichophyton tonsurans* (mostly in the United States) and *Trichophyton rubrum* (Elewski *et al.*, 2008).

The dermatophytes invade the hair shaft in any of two ways i.e., endothrix and ectothrix.

In the former hair shaft is filled with hyphae and spores and arthroconidia is inside the hair shaft only and the cuticle of the hair remains intact. However, in later hyphae and spores cover the outside of the hair shaft causing destruction of the cuticle.

1.1. Prevalence

The epidemiology of TC varies geographically due to socioeconomic, environmental, lifestyle, and climatic conditions (Table 1) shows that Germany has the lowest prevalence and Philadelphia (USA) shows the highest prevalence of TC. The prevalence of TC in a data in tertiary care center of Karachi on 202 patients there age ranged from 1 to 14 years. 9.4% of patients have previous history of TC which was treated and they were symptom free for a period of 6 months to 1 year before presenting again with the disease.

Table 1: Global Prevalence of Tinea Capitis

Countries	Prevalence (%)
Germany	0.1
Barcelona (Spain)	0.23
Italy	0.3
Palestine	1
London (United Kingdom)	2.5
Tanzania	7.1
Cleveland (USA)	13
Philadelphia (USA)	14

The prevalence of TC in African, Asian, European and North American countries (Balci *et al.*, 2014)

40.1% of the patient's siblings were also suffering from TC and the most prevalent species were anthropophilic. 11.9% of the

patient had contact with household animals like goats and cattle. Majority of the patients belonged to low socio-economic class and only 2% were from higher socio-economic class (Farooqi *et al.*, 2014).

However in Europe *T. tonsurans* (Anthropophilic) is the most common cause of TC since mid-1990s in London (Tan, 2005) and its incidence is increasing in different parts of the world including USA. (Foster *et al.*, 2004) Another microorganism is *M. canis*, a zoophilic dermatophyte is transmitted from household pets and infections resulting from it are usually severe with erythema and pustules. The incidence of *M. canis*, a zoophilic dermatophyte is also increasing in central and southern Europe. The common microorganism causing infection in East Africa and the Indian sub-continent is *T. violaceum* (Moriarty *et al.*, 2012).

Children are particularly susceptible to dermatophytic infections because of their poor personal hygiene habits and poor environmental sanitation and hence most frequent in them particularly in boys under 12 years of age. It has also been linked with their frequent visit to the hairdresser as shorter hair also facilitates easy access for circulating spores (Friedlander *et al.*, 2003). *M. canis* infection is prevalent in boys, while boys and girls are equally infected by Trichophyton species (Aly, 1999). TC is more common in younger children as compared to adults this was due to the presence of *Pityrosporum orbiculare* (*Pityrosporum ovale*), which is part of normal flora, and also from the fungistatic properties of fatty acids of short and medium chains in post-pubertal sebum due to which adults are rarely infected (Gorbach *et al.*, 1997).

TC may occur sporadically or epidemically and its prevalence varies throughout the world with a rise over the last few decades (Kundu *et al.*, 2012).

2. MATERIALS AND METHODS

2.1. Case History

An 11 year old boy visited Shifa ul Mulk Memorial hospital, Hamdard University Karachi on April 2013. Initially, he was interviewed regarding demographic data such as age, duration of disease, socio-economic group and living conditions particularly sharing of towels, combs, having pets at home, fungal infections among siblings and any prior antifungal treatment. On physical examination particularly the scalp there were signs of severe scaly, crusting with bald patches, indicating severe manifestation of TC. The patient was suffering from this complain since 3 months that gradually aggravated and worsen on the 5th month when he visited the OPD. He did not receive any prior treatment and hence was a naïve patient. Furthermore, enlarged post auricular lymph nodes were noticeable with body temperature of 100°F. However, there was no other past medical history with normal progression of childhood development.

2.2. Laboratory Investigation and Diagnosis

The patient was referred to DOW Laboratory, Karachi for identification of the microbial infection. The laboratory highlighted that hair samples were collected by sterile hairbrush technique, while skin scrapings were cultured on Sabouraud's agar for the identification of microbes. The clinical report of skin scrapings showed fungal hyphae which was identified as *T. violaceum* and the case was diagnosed as TC.

2.3. Treatment

A photo was taken before treatment (Fig. 1a), followed by the prescription of Unani product as Nuskha No. 9 (Table 2a). He was instructed to use one sachet per day (half in the

morning and half in the evening) in a cup of boiling water as decoction. Additionally, Nuskha No. 10 (Table 2b) was also prescribed as 1 tablet per day before bed-time. The patient was advised for follow-up after every 2-weeks and treatment was continued for a period of 8-weeks.

3. RESULT AND DISCUSSION

The patient, a 11 year old boy suffering from TC which is a fungal infection caused by *T. violaceum*. This appears to be a predominant pathogen in children and adults with multiple clinical variations globally (Table 1) including Pakistan (Farooqi *et al.*, 2014) and India (Moriarty *et al.*, 2012).

The major complaint by the patient was itching along with hair loss accompanied by dull grey patches. These were the most common clinical signs and symptoms of TC and are in accordance with the descriptions reported earlier (Kundu *et al.*, 2012). After 2-weeks of treatment with polyherbal Unani medicine i.e., Nuskha No. 9 and No. 10 a slight improvement was noted and patient remarked that the itching, secretions and crusting has been reduced. It was reflected by the reduction in the abrasions on scalp which are usually produced by continuous scratching and thus advised to continue the treatment. Upon 2nd follow-up visit after 4-weeks of treatment (Fig. 1b) a significant improvement was observed and the treatment was continued for another 4-weeks. A significant improvement was noted followed by normal hair growth in the affected area (Fig. 1c).

Lymphadenopathy, was also noted which has been reported in other cases of TC appearing as painful nodes that enlarges with a passage of time possibly due to immunological events particularly occurrence of inflammatory processes in the area drained by the lymph nodes (Karpf, 1990).

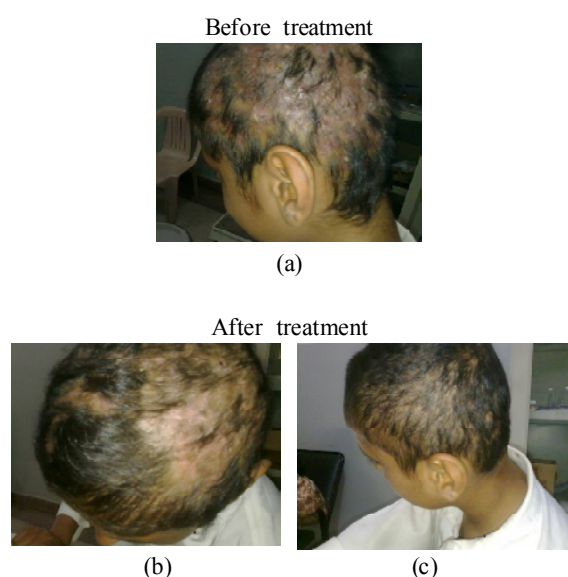


Fig. 1: Effect of Nuskha No. 9 and tablet Nuskha No. 10 used against Tinea capitis

a) A 11 years old boy with TC; b) After 1 month treatment with Unani formulation shows marked improvement in the scalp condition; c) Follow up visit after 2 months from the start of the treatment shows complete cure and normal healthy hair growth.

The dermatophytes usually invade non-living keratinized layer of skin, stratum corneum and use keratin as a nutrient source by producing keratinase enzyme that facilitates its invasion. At the site of infection inflammatory reaction induces redness, swelling, heat or burning and finally alopecia (Sultana *et al.*, 2011).

Both the Unani formulations (Nuskha No. 9 and No. 10) containing medicinal plants (9 herbs in Nuskha No. 9 and 4 herbs in Nuskha No. 10) are reported to possess diverse pharmacological properties particularly anti-bacterial, anti-fungal and anti-inflammatory. These plants are known for diverse pharmacological actions were used against many diseases including skin infections and for blood purification.

For example, in Nuskha No. 9, Fumaria (shahtara) contain fuyuziphine, an alkaloid with antifungal activity (Sarma *et al.*, 1999); *Sphaeranthus indicus* enhance the process of wound healing comparable to neomycin (Sadaf *et al.*, 2006); *Terminalia chebula* exhibiting significant anti-oxidant activity along with steroids and flavonoids (Aruna *et al.*, 2012) and hence used in treating various skin diseases and *Rosa damascena* having anti-inflammatory related to vitamin C which also has antioxidant effects (Hajhashemi *et al.*, 2010).

Likewise, in Nuskha no 10 the analgesic property of *A.leucophloea* was due to the blockade of prostaglandin release or synthesis or other endogenous substances that excite pain nerve endings (Juan, 1979). *Cassia absus* is also useful in fungal skin infection and other skin disease (Ahmed *et al.*, 2011).

Other poly herbal antifungal formulation including Melicon V ointment (www.poulvet.com) and candigone capsules (www.renewlife.com/candigone.html) contain *S. chirayita* extract and berberry root extract which are also present in Nuskha No. 9 and 10, respectively.

The conventional medicine are also available for treating TC e.g griseofulvin, is fungistatic and inhibits the mitosis of dermatophytes by interacting with microtubules and disrupting the mitotic spindle (Roberts *et al.*, 2005). It is effective against trichophyton, microsporum and epidermophyton. Griseofulvin in school screening programmes and almost eradicated TC as an endemic condition in the developed world in the 1950s, but it re-emerged as a public health concern in the United Kingdom in the 1990s in school children (Hay *et al.*, 1996). Increased mass tourism and mobile populations may also have contributed to the changing epidemiological trends (Havlickova *et al.*, 2008). The griseofulvin shows many adverse effects such as headaches and

Table 2a: Constituents of Nuskha No. 9 Used Against TC

Botanical name	Family	Local name	Unani name	Medicinal use	Dosage/gm	Part used
<i>Swertia chirayita</i>	Gentianaceae	Indian Gentian	Chirayta	Blood purifier (Skin diseases)	6	Bark
<i>Fumaria indica</i>	Fumariaceae	Indian Fumitory	Shahrtara	Blood purifier	6	Leaves
<i>Tephrosia purpurea</i>	Leguminosae	Wild indigo	Sarphoka	Blood purifier	6	Bark
<i>Sphaeranthus indicus</i>	Compositae	East India Globe Thistle	Gul mundi	Anti-inflammatory, Wound healing activity	6	Fruit
<i>Ziziphus jujuba</i>	Rhamnaceae	Jujube	Unab	Blood purifier	6	Fruit
<i>Santalum album</i>	Santalaceae	White Sanda	Sandal safaid	Blood purifier	6	Wood
<i>Terminalia chebula</i>	Combretaceae	Chebulic Myrobalan	Halyela siyah	Digestive Tonic	6	Fruit
<i>Tamarix dioica</i>	Tamaricaceae	Tamarisk	Jhao	Liver tonic	6	Leave
<i>Rosa damascena</i>	Rosaceae	Damask rose	Gul e surkh	General tonic	6	flower

Table 2b: Constituents of Tablet Nuskha No. 10 Used Against TC

Botanical name	Family name	Local name	Unani name	Activity	Dosage/gm	Part used
<i>Berberis aristata</i>	Berberidaceae	Berberry	Rasoot	Blood purifier	3	Resin
<i>Curcuma zedoaria</i>	Zingiberaceae	White turmeric	Nar kachur	Blood purifier	3	Rhizome
<i>Cassia absus</i>	Fabaceae	Jasmeejaz	chaksu	Blood purifier	3	Seed
<i>Acacia leucophloea</i>	Fabaceae	White Catechu	Kath safaid	Anti-pyretic	3	Resin

gastrointestinal disturbances and sometimes it needs to be discontinued. Severe allergic reactions, hepatic toxicity and leucopenia rarely occur; therefore routine blood studies are not necessary unless treatment is to last for many months or the dosage is exceptionally high. It is contraindicated in children with porphyria, lupus erythematosus, or severe liver disease (Higgins *et al.*, 2000)

Terbinafine is also fungicidal and acts by inhibiting squalene epoxidase, a membrane-bound enzyme in the biosynthetic pathway of sterol synthesis of the fungal cell membrane. It is well absorbed and binds strongly and nonspecifically to plasma proteins. (Gupta *et al.*, 2003)

Also orally itraconazole is effective in TC infections against Trichophyton and Microsporum. Depending on its concentration it exhibit both fungistatic and fungicidal properties by inhibiting the cytochrome P-450-dependent enzymes and synthesis of ergosterol (the principal component of fungal cell membranes), respectively. (Gupta *et al.*, 1998).

The Unani formulations are cost effective as from Nuskha No. 9 with 5 sachets (10 gm each) cost approx Rs.1/sachet and total cost for entire treatment (8 weeks) is around Rs.45. However, Nuskha no 10 (12 tablets of 500mg) approximately costs Rs.9.0. The cost of 8 weeks treatment is a combination of both used for 8 weeks of treatment cost Rs.94 approx. (0.8971\$). On the other hand, Grisol 500 mg (Lisko Pharma) Rs.10 per tablet used for a period of 8 weeks with total cost of treatment is around Rs.440 (4.20\$). Thus Unani formulation is 4.6 times less costly than conventional medicine.

It is concluded the nuskha No. 9 and 10 are effective against TC, it is cost effective, however more data is required to justify its efficacy, safety and its mechanism of action.

Author's Contribution and Declaration

Leena Hameed Afridi: Conducted the trial.

Tasneem Qureshi: Supervised the research study

The authors declare that there is no conflict of interest.

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Phytochemical and Dermatological Investigations of *Tribulus terrestris* L.

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Abstract

Tribulus terrestris extracts were prepared using solvents of different polarities and their irritant inducing effects were assessed *in vivo* on rabbit's ears. The result indicates that the polar components were in higher yield than the others and amounts of the extracted material were directly proportional to the polarities of solvents. Preliminary phytochemical screening shows diversity of compounds and comparative TLC analytical behaviour further indicated the resolution of mostly five compounds from the non-polar, intermediate polar and highly polar materials by different solvent systems. Irritant potency of water extract was assessed on rabbit's ears using a known method that was proved to be the most potent irritant (with +++ response, propagated in an area of 2.70 cm² diameter).

Keywords

Phytochemical screening, Irritancy, Chromatography, *Tribulus terrestris*.

1. Introduction

Large numbers of plants have been used globally as sources of medicine and these

plants preparations often referred as "Herbal drugs" or "Herbal remedies" have been playing appreciable role in keeping human health and improving the quality of human life for chiliads of years (Craig, 1999, Roberts, 1988, Tripathi and Tripathi, 2003). Now-a-days the application of herbal drugs in the world especially Europe and in America is growing rapidly for adjusting the imbalances induced by consumption of modern diets and sedentary lifestyles (Balandrin et al., 1985, Culture, 1996). Many plants have been reported to contain phytochemicals which causes skin irritation. These chemicals often occur in leaves, stems, bark, flowers and fruit etc. of certain plants. Some of them are discharged only when the plant parts are crushed, while the others exudates on their surfaces which cause dermatitis by their direct action (Evans and Schmidt, 1980, Basketter et al., 2004, Slodownik et al., 2008). Many plants that give rise to irritant reactions are ordinary house plants, garden plants or edible fruits and seeds etc. (Wattanakrai et al., 2007, Evans and Schmidt, 1979).

Tribulus terrestris L., a weed of tillable land, gardens widely distributed worldwide as well as in warm dried areas of Pakistan and

commonly known as calatrops, small-caltrops, land-caltrops, puncture-vine in English and bhakhra, bhakhrou, gokhru and chota-gokhru in different local languages. The plant is a prostrate branched annual or biennial herb, silky hairy with dragging stems (Plucknett and Holm, 1977). Leaflets are sub-equal, oblong, mucronate very variable in size. Flowers are yellow on peduncle shorter than the leaves. Its phytochemistry is well established and contains: Furostanol and spirostanol saponins of tigogenin, neotigogenin, gitogenin, neogitogenin, hecogenin, neohecogenin, diosgenin, chlorogenin, ruscogenin, sarsasapogenin (Kostova and Dinchev, 2005, Alavia *et al.*, 2008, Simeonov *et al.*, 2011), quercetin 3-O-glycoside, quercetin 3-O-rutinoside, kaempferol 3-O-glycoside, quercetin and kaempferol (Alavia *et al.*, 2008), methylprotodioscin, protodioscin (Kostova *et al.*, 2002), arboline, tribulusterine (Bremner *et al.*, 2003) rhamno-galact-uronan (Chen *et al.*, 2002) and tribufurosides G and H (Xu *et al.*, 2009). *T. Terrestris* is used traditionally as diuretic, antiseptic, anti-inflammatory for the mucus membrane of urinary tract, demulcent, tonic, emmenagogue, also used for spermatorrhoea, phosphaturia, dysuria, impotence, in chronic cystitis, calculus affections, gonorrhoea, painful micturition and in chronic kidney inflammation, reduces the high blood pressure, cholesterol, against erectile dysfunction, increases the testosterone level, fertility, libido and possesses anti-oxidant properties (Neychev *et al.*, 2007, Adaikan *et al.*, 2000, Chu *et al.*, 2003, Gauthaman *et al.*, 2002, Gauthaman *et al.*, 2003).

Our study on *T. terrestris* L. presents the separation and identification of the active irritant principles residing in it through solvent extractions and its dermatological effects on rabbit's ears.

2. Materials and Method

2.1. Chemicals

Silica gel (60 F₂₅₄ Merck, Germany), organic solvents (BDH (Germany) and all other chemicals were of analytical grade and were purchased from the local market.

2.2. Animals

Healthy adult male/female rabbits (1-1.5 kg) of albino strain of species *Oryctolagus cuniculus* were purchased from the local market. These animals were acclimatized in the animal house of the University College of Pharmacy, University of the Punjab, Lahore, for a period of 3 days. They were provided with carrots, fresh green fodder (clover) and tap water *ad libitum*.

2.3. Plant Collection and its Processing

T. terrestris L. (Fig. 1) was collected from the botanical garden of Government College University, Lahore Pakistan and was authenticated by Prof. Dr. Zaheer-ud-din khan, Department of Botany, with voucher no GC 1970 and specimen was deposited in the herbarium of Pharmacognosy section, University College of Pharmacy, University of the Punjab, Lahore. The plant was dried under the shade at room temperature for about 15 days then pulverized to a fine powder and stored in amber coloured bottles. The pulverized dried plant (100 g) were separately soaked and extracted with 500 mL of hexane, light petroleum ether (40-60°), chloroform, dichloromethane, ethyl acetate, acetone, ethanol, methanol and water. Maceration was carried out in each solvent for 7 days at room temperature (27±2.5°C) followed by its removal under reduced pressure and the residues were weighed and stored.



Fig. 1: *Tribulus terrestris* L.

2.4. Preparation of Dilutions

Dried extracted material of each sample was constituted as 20 mg/ml (w/v) solution with acetone. The dilution series was prepared according to the equation (Evans and Schmidt, 1979).

$$C_m = C_o \times a^{-m}$$

Where, C_o = initial concentration, C_m = Concentration after m dilution, a = Dilution factor. The dilution factor in all the cases was kept at 2 and six or seven dilutions were prepared.

2.5. Phytochemical Screening

The condensed water extract were used for the preliminary screening of phytochemical

for the identification of main constituents present in the dried powdered materials (Purohit *et al.*, 2007).

2.6. Thin Layer Chromatography

It was performed using silica gel using different solvent systems for petroleum ether, chloroform and methanol extracts and compounds were visualized using UV light and iodine vapors on TLC chromatogram (Stahl, 1969).

2.7. Irritancy Assay

The biological assay for irritancy was performed as described earlier (Evans and Schmidt, 1979). Group of 6 rabbits for each dilution was used and a 10 μ l sample of the most diluted solution was applied to one of the ear of a rabbit while untreated ear served as a control. The other group of animals were treated similarly by increasing concentration of irritants. Rabbits were examined after 30 minutes of its application with 30 minutes intervals. The number of ears showing marked inflammation of the major blood vessels was recorded. The irritant dose (ID₅₀) corresponds to the 50 % cumulative frequency and the evaluation of irritant response was determined using Hacker method (Busch, 1967).

3. Results And Discussion

T. terrestris L. is a herbaceous or somewhat low shrubby plant often found as weed in the fields of economically important crops (Plucknett and Holm, 1977, Reddi, 1981, Boydston, 1990) causing significant damages. It was a matter of familiar observation that during the removal, it causes irritation on the hands of workers (Zhai and Maibach, 2004, Mitchell and Rook, 1977, Calnan, 1975). Moreover, during the collection of plant fine trichomes, present on the lower surface of

the leaves and on its spiny fruits produced irritation on the flexor side of collector's hands.

One of the important dermatitis parameters is irritancy which is very common and could easily be appraised in animals by observing a rapid reaction of rabbits against the solvent extracts. Previously, many authors separated, characterized and evaluated the structure-activity relationship of some of the allergenic/irritants including alkaloids, saponins, flavonoid glycosides, terpenoids and similar phytochemicals compounds from various members of different the families (Misra, 1962, Athar and Mahmood, 1985, Alavia *et al.*, 2008, Italo *et al.*, 2009, Ntalli *et al.*, 2010). In Table 1 percent yield of both non-polar and polar ingredients are presented. The polar components extracted in ethanol (25.42%), methanol (30.72%) and water (63.52%) were in higher yield. However, low yields of non-polar components in petroleum

Table 1: Percent Yield of the Extracted Materials Using Different Solvents

S.No.	Solvent	Percentage yield
1.	Hexane	3.26 %
2.	Petroleum ether	5.24 %
3.	Chloroform	10.37 %
4.	Dichloromethane	11.16 %
5.	Ethyl acetate	10.75%
6.	Acetone	15.90 %
7.	Ethanol	25.42 %
8.	Methanol	30.72 %
9.	Water	63.52 %

ether (5.24%) and hexane (3.26%) were also obtained. On the other hand, the components with intermediate polarities were extracted in chloroform (10.37%), dichloromethane (11.16%), ethyl acetate (10.75%) and acetone (15.90%) in intermediate quantities. Thus *T. terrestris* contains a larger proportion of high and intermediate polar compounds than its non-polar components.

Table 2: Preliminary Phytochemical Analysis of Aerial Parts of *Tribulus terrestris* L.

Phyto-constituent	Test	<i>T. terrestris</i>
Alkaloid	Mayer's reagent Replace test with reagent in all	+++
	Wagner's test	+++
	Hager's test	+++
	Dragendroff's test	+++
Glycoside	Fehling's test	+
Tannin	Ferric chloride test	++
Flavonoid	Alkaline reagent test	+
Steroid and triterpenoid	Salkowski test	+
	Lignin	Phloroglucinol test
Saffranine test		+
Amino acid	Ninhydrine test	-
Carbohydrates	Molisch's test	-
Fats and fixed oil	Copper sulphate test	-

Table 3: Comparative TLC Analysis of Petroleum Ether Extract of *T. Terrestris* L.

Solvent Systems		No. of Comp.	Detection		hRf values
Solvents	Ratio		UV light	Iodine	
P.E./CHCl ₃	90:10	3	blu, blu, pur	l-yel, yel, yel	21, 41, 52
P.E./CHCl ₃	80:20	4	gry, yel, pur, blu	l-yel,yel,yel,l-yel	14, 21, 61, 70,
P.E./CHCl ₃	70:30	3	yel, gry, blu	l-bro,yel,yel	29, 40, 81
P.E./CHCl ₃ /MeOH	90:10:2	5	red, blu, yel, pin, blu	l-yel,l-el,yel,yel,yel	26, 35, 55, 68, 83
P.E./CHCl ₃ /MeOH	90:10:5	5	blu, pur, pur, yel, blu	yel,yel,yel,l-yel,bro	8, 24, 36, 41, 66
P.E./CHCl ₃ /MeOH	80:20:2	4	blu, pur, blu, blu	l-yel,yel,yel,l-bro	18, 47, 68, 85
P.E./CHCl ₃ /MeOH	80:20:5	5	blu, pur, blu, blu, pur	l-yel,yel,yel,yel,d-bro	27, 58, 67, 72, 82
P.E./CHCl ₃ /MeOH	80:20:10	5	pin, pur, yel, pin, bro	yel,l-yel,yel,yel,d-bro	11, 30, 65, 79, 83
P.E./CHCl ₃ /MeOH	70:30:2	5	yel, blu, gry, pur, gry	yel,l-yel,yel,yel,d-bro	9, 28, 56, 77, 89
P.E./CHCl ₃ /MeOH	70:30:5	5	bro, yel, blu, pin, yel	yel, l-yel, yel, yel, d-bro	20, 65, 75, 82, 89
P.E./CHCl ₃ /MeOH	70:30:10	4	blu, yel, blu, red	yel, l-yel, yel, l-bro	24, 57, 68, 89
P.E./CHCl ₃ /MeOH	60:40:1	4	blu, yel, pin, blu	l-yel, l-bro, yel, yel	20, 54, 62, 78
P.E./CHCl ₃ /MeOH	60:40:2	3	blu, yel, blu	l-yel, l-yel, bro	28, 65, 76
P.E./CHCl ₃ /MeOH	60:40:5	1	blu	l-yel	81
CHCl ₃ /MeOH	10:20	2	gre, blu	yel, yel	64, 76
CHCl ₃ /MeOH	15:25	3	yel, blu, red	yel, d-yel, l-yel	65, 74, 80
CHCl ₃ /MeOH	20:30	3	yel, blu, red	l-yel, yel, d-yel	65, 70, 82
CHCl ₃ /MeOH	25:35	3	yel, blu, red	l-yel, d-yel, yel	77, 83, 87
HCl ₃ /MeOH/HCl	30:40:1	2	yel, blu	yel, bro	75, 73
HCl ₃ /MeOH/HCl	30:40:2	2	yel, blu	l-yel, d-yel	76, 80
HCl ₃ /MeOH/HCl	40:50:3	2	pur, yel.	l-yel, d-yel	56, 78
HCl ₃ /MeOH/HCl	35:55:5	2	pur, l-blu	l-yel, d-yel	60, 72

Table 4: Comparative TLC Analysis of Chloroform Extract of *T. Terrestris*

Solvent Systems		No. of Comp.	Detection		hRf values
Solvents	Ratio		UV light	Iodine	
P.E./CHCl ₃ /MeOH	90:10:2	4	red, red, gre, pin	d-yel, yel, bro, yel	35, 59, 65, 79
P.E./CHCl ₃ /MeOH	90:10:5	3	yel, gre, gre	bro, d-yel, d-bro	24, 37, 64
P.E./CHCl ₃ /MeOH	80:20:2	4	org, gre, gre, pin	l-yel, d-bro, bro, yel	35, 45, 53, 68
P.E./CHCl ₃ /MeOH	80:20:5	3	pin, bro, gre	bro, yel, d-bro	34, 57, 73
P.E./CHCl ₃ /MeOH	80:20:15	4	pin, pin, gre, blu	l-yel, yel, d-yel, yel	61, 71, 75, 87
P.E./CHCl ₃ /MeOH	70:30:1	3	gre, pin, blu	l-yel, yel, yel	10, 25, 67
P.E./CHCl ₃ /MeOH	70:30:2	3	gre, pin, blu	l-yel, yel, yel	9, 25, 67
P.E./CHCl ₃ /MeOH	70:30:5	4	yel, gre, yel, blu	d-yel, l-yel, yel, yel	8, 20, 47, 64
CHCl ₃ /MeOH	95:5	4	yel, gre, gre, pin	l-yel, yel, d-yel, yel	41, 56, 72, 79
CHCl ₃ /MeOH	90:10	2	blu, pin	l-yel, d-yel	74, 81
CHCl ₃ /MeOH	85:15	2	blu, pin	l-yel, d-yel	76, 84
CHCl ₃ /MeOH	80:20	1	gre,	bro	88
CHCl ₃ /MeOH/HCl	80:20:1	3	pin, yel, blu	l-yel, yel, yel	21, 46, 68
CHCl ₃ /MeOH/HCl	75:25:2	4	gre, pin, yel, blu	l-yel, yel, yel, yel	42, 65, 76, 81
CHCl ₃ /MeOH.HCl	70:30:3	4	gre, gre, yel, blu	l-yel, yel, yel, yel	14, 29, 73, 85
CHCl ₃ /MeOH/HCl	65:35:5	4	org, gre, pin, blu	yel, l-yel, yel, yel	9, 17, 32, 64
CHCl ₃ /MeOH/AcA	90:15:3	4	org, gre, pin, pin	yel, yel, d-yel, yel	20, 44, 58, 69, 80
CHCl ₃ /MeOH/AcA	80:20:5	3	blu, yel, gre	d-bro, d-yel, yel	21, 50, 68
CHCl ₃ /MeOH/AcA	70:30:10	3	gre, yel, blu	d-yel, yel, l-yel	33, 42, 54
CHCl ₃ /MeOH/AcA	65:35:12	4	org, pin, gre, blu	d-yel, l-yel, yel, l-yel	71, 56, 77, 26

Table 5: Comparative TLC Analysis of Methanol Extract of *T. Terrestris* L.

Solvent Systems		No. of Comp.	Detection		hRf values
Solvents	Ratio		UV light	Iodine	
CHCl ₃ /MeOH	95:5	2	pin, blu	—	39, 40
CHCl ₃ /MeOH	90:10	2	d-pin, pin	—	25, 51
CHCl ₃ /MeOH	85:15	2	d-pin, pin	—	9, 22
CHCl ₃ /MeOH	80:20	5	pin, org, pin, pin, blu	l-yel, yel, yel, yel, l-yel	9, 16, 36, 65, 77
CHCl ₃ /MeOH	75:25	4	org, pin, gre, pin	d-bro, l-yel, l-yel, yel	10, 16, 49, 63
CHCl ₃ /MeOH	70:30	5	gre, org, pin, pin, blu	l-yel, yel, yel, l-yel, yel	19, 27, 37, 57, 87
CHCl ₃ /MeOH	65:35	3	pin, d-pin, blu	l-yel, d-yel, yel	9, 23, 54
CHCl ₃ /MeOH	60:40	4	pin, d-pin, red, blu	l-yel, d-yel, yel, yel	30, 34, 53, 70
MeOH/AcA	95:5	1	yel	yel	72
MeOH/ AcA	90:7	1	yel	yel	75
MeOH/ AcA	90:10	1	l-yel	l-yel	78
MeOH/ AcA	85:7	1	l-yel	l-yel	70
MeOH/ AcA	85:10	2	gry, gre	bro, bro	53, 69
MeOH/ AcA	80:15	2	gry, gre	bro, d-bro,	54, 69
MeOH/ AcA	75:20	2	gry, gre	yel, d-bro	43, 57
MeOH/ AcA	70:30	1	gre	d-bro	55
MeOH/HCl	95:10	2	gry, gre	l-bro, d-bro	43, 65
MeOH/HCl	90:15	1	blu	yel	18
MeOH/HCl	85:30	4	yel, gre, pin, d-pin	d-bro, d-yel, yel, yel	22, 34, 57, 89
MeOH/HCl	80:30	2	pin, yel	yel, l-yel	20, 47,
MeOH/HCl	80:35	2	d-pin, d-yel	l-bro, l-yel	25, 56
MeOH/HCl	75:30	2	l-pin, l-yel	l-bro, l-yel	20, 41

Where: P.E. = Petroleum ether, CHCl₃ = Chloroform, MeOH = Methanol, EtAc = Ethyl acetate, AcA = Acetic acid, blu = blue colour, yel = yellow colour, gre = green colour, pur = purple colour, pin = pink colour, gry = grey colour, red = red colour, l-yel = light yellow, d-yel = dark yellow, l-bro = light brown, d-bro = dark brown, org. = orange colour, d-pin = dark pink.

**Table 6: Irritant Response of Different Doses of the Water Extract of
T. Terrestris on Rabbit's Ears**

Doses g/ml	Responses after – Acute time												Chronic time		
	30 min	1h	2h	3h	4h	5h	6h	7h	8h	9h	11h	12h	24h	48h	72h
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	±	±	±	±	±	-	-	-
20	-	+	+	+	+	+	+	+	++	+	+	+	-	-	-
25	-	+	+	++	++	++	++	++	++	++	++	++	+	±	±
30	+	++	++	++	++	++	++	++	++	++	++	++	+	+	±
35	+	++	++	++	++	++	++	++	++	+++	+++	+++	+	+	±
40	+	++	++	++	++	+++	+++	+++	+++	+++	+++	+++	+	+	+
45	+	++	++	++	++	+++	+++	+++	+++	+++	+++	+++	+	+	±
50	+	++	++	++	++	+++	+++	+++	+++	+++	+++	+++	+	±	-
55	+	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+	-	-
60	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+	+

Where: - = No reaction, ± = doubtful reaction, + = Slight reddening of the main vessels, ++ = Marked reddening of the main vessels, +++ = Intense reddening of the entire ear.

Three solvent extract samples were analysed by comparative TLC, using 22 different solvent systems. The main intention of this analysis was to have an estimation of total number and chromatographic behaviour of the ingredients residing in each extract. The most appropriate solvent system which resolved the mixture of petroleum ether extract of the *T. terrestris* materials into five or more components, appeared to be petroleum ether/chloroform/methanol (90:10:2; 90:10:5; 80:20:5; 80:20:10 and 70:30:2) (Table 2). Chloroform extract was segregated maximally into four components by eight or ten different solvent systems but was not further resolved (Table 3). The mixture of polar components, present in the methanol extract of samples was resolved into five major compounds by chloroform/methanol (80:20; 70:30; 65:35 and 60:40) (Table 4). Water extract was used for the irritancy assay because in preliminary irritancy assay it was most active as compared to other extracts probably, the elements residing in these mixtures were unable to penetrate the skin to induce deep damage to the epidermal tissues, but strong enough to produce dilation of blood vessels and superficial redness (Anderson *et al.*, 2009, Basketter *et al.*, 2004). The water extracts seems to contain the most active mixture of phytochemical compounds than the other solvent extracted materials. Eight out of eleven water extracts of *T. terrestris* were dermatologically active on rabbit's ears and their irritant responses was dose dependent (10 to 60 mg/ml) as reflected by its severity and duration on the animal ears (Table 5). It produced commemorated erythema on rabbit's ear after one hour of application and in most cases this reaction persisted for about 48 hours (Table 6). The polar nature of penetrated

substance (s) most likely interacted with the cell membrane and cellular contents of superficial and deeper layers of epidermis inducing release of inflammatory mediators in superficial and deeper layers epidermal layers causing damage which was reflected as localized inflammation. Similar strong to moderate irritant reaction have been noted by various compounds (Anderson *et al.*, 2009, Loffler *et al.*, 2001). The isolation of irritant principle(s) from *T. terrestris* exact and its mechanism of action needs to be explored further that will be helpful to design the precautionary measures against it.

Author's Declaration

The authors declare that there is no conflict of interest.

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Inhibitory Effect of *Aegiceras corniculatum* Against Lung Injury in Carrageenan-induced Mouse Pleurisy Model

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Abstract

Aegiceras corniculatum is a mangrove plant traditionally used against rheumatism, pain and inflammation. The present study was aimed to explore the anti-inflammatory activity of methanol and ethyl acetate extracts derived from *A. corniculatum* in carrageenan-induced pleurisy mouse model of acute inflammation. Mice were treated with different doses (10, 50 and 100 mg/kg, i.p.) of methanol and ethyl acetate extracts followed by induction with intrapleural injection of 1% carrageenan (0.1 ml). After 4 hours animals were euthanized and pleural lavage was collected in sterile PBS and myeloperoxidase (MPO), nitrate/nitrite concentration and leukocytes migration were determined. The methanol and ethyl acetate extracts at higher doses (100 mg/kg) demonstrated remarkable reduction ~82% in leukocytes migration. Likewise, MPO and nitrate/nitrite concentrations also showed a dose dependent decline in the presence of both methanol and ethyl acetate extracts reaching maximum value (83%) at 100 mg/kg thus implying its protective effect in the management of acute lung inflammation. These results led us to suggest that carrageenan administration into the pleural cavity caused accumulation of fluid containing infiltrated PMNs from lung tissues as well as a large number of poly-

morphonuclear cells (PMNs) that enhanced the production of interleukin-1 β , IL-6, tumor necrosis factor-5 α and cyclooxygenase (COX-2) which were more likely to be attenuated by the *A. corniculatum* extracts revealing its protective effect in acute lung inflammatory conditions.

Keywords

Aegiceras corniculatum, anti-inflammatory, carrageenan-induced acute lung inflammation.

1. INTRODUCTION

Inflammation is a complex phenomenon which involving multiple biochemical, cellular and molecular processes for activating the host defense and regulating the uncontrolled inflammatory developments causing various inflammatory diseases (Vane *et al.*, 1998; Jiang *et al.*, 2003; Willerson *et al.*, 2004). In inflammatory conditions, oxidative burst of polymorphonuclear neutrophils (PMNs) produce extra- and intra-cellular reactive oxygen species (ROS) or free radicals namely, hydroxyl radical (\bullet OH), hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\bullet-}$) via activation of NADPH oxidase and myeloperoxidase (MPO) enzymatic pathways and protect the body from immune responses. In neutrophil degranulation, myeloperoxidase, a heme-containing peroxidase

which are stored in azurophilic granules of neutrophilic granulocytes are released from the cells and are associated with inflammation and oxidative stress (Goldblum *et al.*, 1985). In phagosome of neutrophils, MPO also plays a vital role in killing of microbes and forming hypochlorous acid (HOCl) from the chlorination cycle. But under adverse circumstances its excessive production oxidizes vital biomolecules such as DNA, proteins, and lipids thereby leading to the development of various diseases including neurodegenerative disorders, cardiovascular diseases and cancer (Selvakumar *et al.*, 2011).

Globally, it is a common practice to treat different diseases with medicinal plant(s). *A. corniculatum*, a mangrove plant growing in the wetland of tropical and subtropical regions of Indus Delta valley of Pakistan (Roome *et al.*, 2008). Its phytochemical and pharmacological evaluation revealed that it is enriched with different metabolites, mainly including flavonoids, polyphenols (Tanin and lignin), sterols, pentacyclic triterpenes (Aegicerin, genin) and its derivatives as well as saponins (Corniculatonin) (Zhang *et al.*, 2005). It possesses anti-diabetic, anti-asthmatic, anti-rheumatic, anti-inflammatory, anti-allergic, antioxidant, and hepatoprotective activities (Roome *et al.*, 2008). In our previous study, we reported that *A. corniculatum* extracts significantly inhibited inflammatory pathways *via* suppressing COX-1, 2 and 5-LOX metabolites as well as antagonized oxidative stress. The anti-oxidant properties of n-hexane, methanol and ethyl acetate extracts were also identified which were associated with a variety of chemical constituents residing in the plant that inhibited the respiratory burst in cells (Roome *et al.*, 2008).

Carrageenans are sulphated linear polysaccharides of d-galactose and 3,6-anhydro-

d-galactose extracted from certain red seaweeds of the Rhodophyceae class are also a common food additive. Its unique chemical structure activates body immune responses, which has been identified as a dangerous invader and causing inflammation (Prajapati *et al.*, 2014). In most animal studies, it is used to induce inflammation (paw or ear edema) for evaluation of anti-inflammatory activities of compounds/drugs and also provide information about their mechanism of action against different pro-inflammatory mediators such as leukotrienes, prostaglandin, bradykinin, histamine, platelet-activating factors, interleukins (ILs) (Saldanha *et al.*, 2016).

Considering anti-inflammatory and anti-oxidant properties of *A. corniculatum*, the present study was designed to investigate its effect in carrageenan-induced pleurisy mouse model to determine the inflammatory response including PMN infiltration, myeloperoxidase activity and release of nitrate/nitrite.

2. MATERIALS AND METHODS

2.1. Animals

Swiss mice either sex (20 to 25 g) acquired from the animal house of Dow University of Health Sciences, Karachi, Pakistan, were used throughout the study. The animals were housed under controlled environment and were provided with standard rodent chow and water. Animal studies were performed in accordance with the declaration of European Community guidelines for the ethical handling and the use of laboratory animals and through the clearance of institutional animal use committee.

2.2. Chemicals

The following chemicals were used: dimethyl sulfoxide (DMSO), λ -carrageenan (Type IV), indomethacin, dexamethasone and rutin Sigma Chemical Company, USA. Solvent

diethyl ether (RDH, Sigma-Aldrich Laboratory Chemicals Germany). Other chemicals including sodium azide and the reagents used in the preparation of PBS and assay buffer were of analytical grade.

All chemicals were dissolved in saline (NaCl, 0.9%,) with the exception of dexamethasone and plant extract that were dissolved in DMSO (10%) while indomethacin were solubilized in 1% Na₂CO₃.

2.3. Plant Material

A. corniculatum were collected during June 2001 from Indus Delta, Sindh, Pakistan at lowest tide and identified by a taxonomist,

Professor Dr. Surriya Khatoon from the Department of Botany, University of Karachi, Pakistan. A voucher specimen representing the collection labeled as KHU G.H.S. No. 68219 has been deposited at herbarium of this department. Its morphological characteristics are presented in Fig. 1.

2.4. Preparation of *A. corniculatum* Extracts

The extracts were prepared with the same procedure as described in (Roome *et al.*, 2008). Air-dried powdered stem (9.0 g) were soaked in 50 L of hexane, ethyl acetate and methanol extracts for 7 days and n-hexane extract (6.0 g or 0.07%, w/w), ethyl acetate extract (8.5 g or

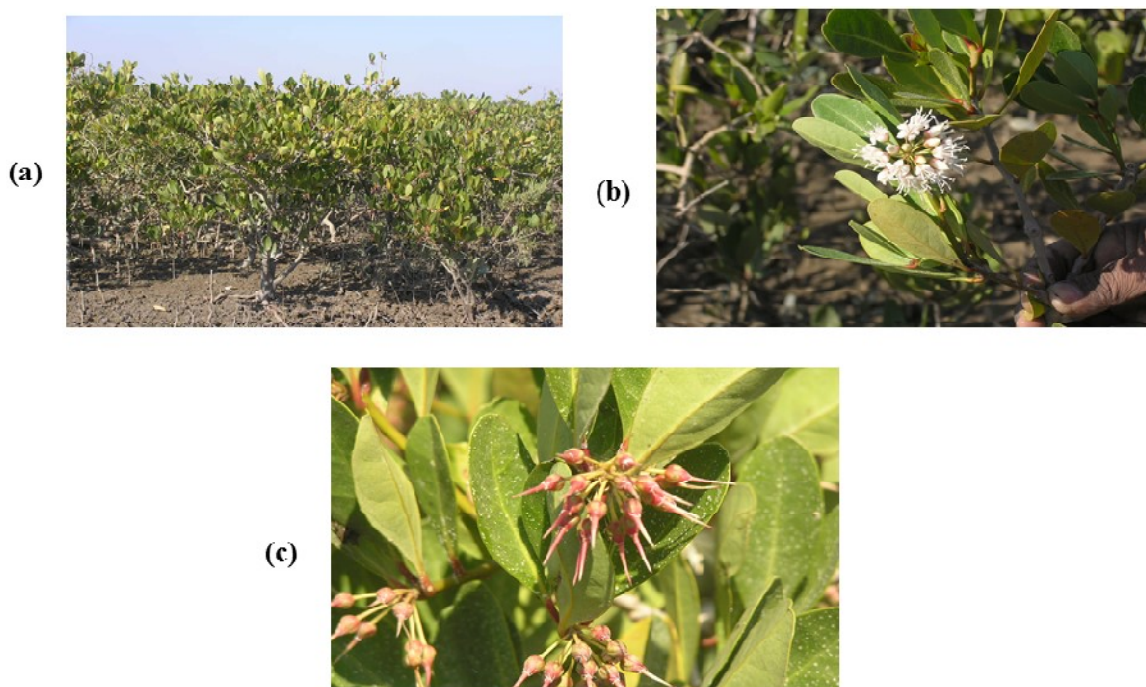


Fig. 1: Morphological characteristic of *Aegiceras corniculatum*

(a) *A. corniculatum* is a shrub or small tree, 1.5-7 m tall usually gregarious and glabrous. (b) Leaves are obovate-oblong to obovate, 4-8 cm long, 2.5-4.0 cm broad, pale green, shining above, glaucous beneath with petiole 3-6 mm long. Flowers are 1.5 cm across usually white and fragrant. (c) Fruit are cylindrical, 2.5-5.0 cm long, 4-6 mm broad and reddish brown (Jafri *et al.*, 1975).

0.09%, w/w) and methanol extract (9.8 g or 0.11%, w/w) were obtained. These extracts were prepared in collaboration with Professor Muhammad Iqbal Choudhary's research group at International Center for Chemical and Biological Sciences, University of Karachi, Pakistan.

2.5. Carrageenan-induced Pleurisy

Swiss mice weighing 20-25 g were divided into groups (n=6) pretreated intraperitoneally with methanol and ethyl acetate extract (10, 50 and 100 mg/kg) derived from *A. corniculatum* or indomethacin (1, 5 and 10 mg/kg) followed by intra-pleural injection of 1% carrageenan (0.1 ml). After 4 hours mice were euthanized, thorax opened and washed with sterile phosphate buffered saline (pH =7.27) and samples of pleural lavage were collected for the determination of leukocytes count, myeloperoxidase and nitrate/nitrite concentrations. Total infiltrated leukocytes count and differential count in pleural fluid were determined using a hemocytometer and observed under light microscope.

2.6. Determination of Myeloperoxidase (MPO) Activity

Different concentrations of MPO (from human neutrophils) used as a standard samples. Pleural aliquot (40 μ l) and standard (dexamethasone) or test agents were transferred into reaction cuvettes followed by addition of assay buffer (0.167 mg/ml of *o*-dianisidine 2HCl and 0.0005% H₂O₂) for the initiation of reaction and stopped by sodium azide (1%). Afterwards, the samples were centrifuged at 50 \times g for 5min, the supernatants were separated and the rates of changes in absorbency were determined spectrophotometrically at 450 nm and expressed as mU/ml (Dalmarco *et al.*, 2002).

2.7. Determination of Nitrate/Nitrite Concentration

Nitrate and nitrite levels in pleural fluid which is an indicator of nitric oxide production was determined by using Griess method (Sautebin *et al.*, 1998). The pleural lavage and equal volume of fresh Griess reagent were mixed and incubated for 10 min at room temperature, followed by its quantification (μ M) at 540 nm spectrophotometrically (Dalmarco *et al.*, 2002).

2.8. Statistical Analysis

Statistical differences between groups were determined by analysis of two-way ANOVA with Dunnet's tests. Less than 0.05 p-value was considered as significant.

3. RESULTS AND DISCUSSION

The carrageenan induction into the pleural cavity induces lung injury which is characterized by cellular infiltration, edema, alveolar thickness, myelin bodies, and appearance of large vacuoles along with enhanced pro-inflammatory cytokine levels, COX-2 and NOS (Cuzzocrea *et al.*, 1999). The present investigation showed that 1% carrageenan (0.1 ml) administered into the pleural cavity of mice caused an acute inflammatory response leading to accumulation of fluid containing a large amount of PMNs. The methanol extract of *A. corniculatum* elicited dose dependent (10, 50 and 100 mg/kg) decline by 35%, 65% and 80 %, respectively. Likewise, at the similar doses, ethyl acetate extract also inhibited infiltration of cells into the pleural cavity by almost similar magnitude (40%, 64% and 84 %). Indomethacin (1, 5 and 10 mg/kg) used as a positive control also reduced infiltration of PMNS as shown in Fig. 2 as compared to the control group.

The effect of *A. corniculatum* on cell influxes were also determined by measuring

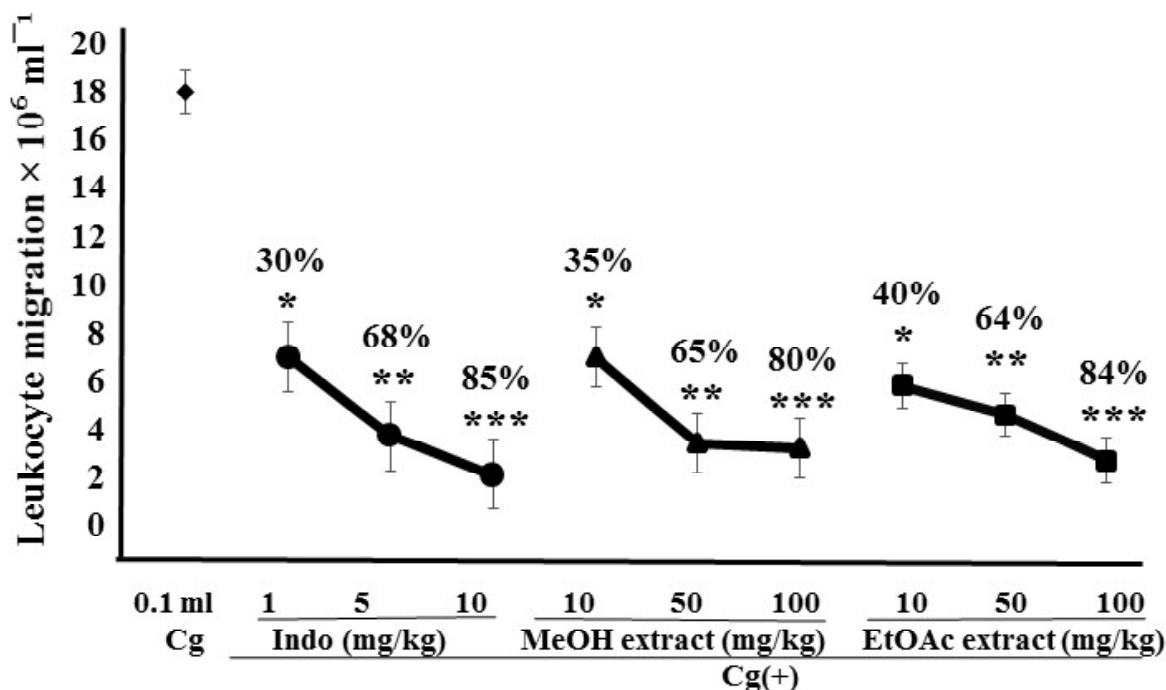


Fig. 2: Effect of *A. corniculatum* extracts on migration of leukocytes in carrageenan-induced pleurisy model

Methanol (MeOH, ▲), ethyl acetate (EtOAc, ■) extracts (10, 50 and 100 mg/kg) and indomethacin (Indo ● 1, 5 and 10 mg/kg) were administered in mouse pleurisy model induced by carrageenan (1 %, 0.1 ml) and leukocyte migration at the site of inflammation was compared with the carrageenan alone (Cg ◆).

The leucocyte count in control (saline treated animals = 2.1±0.6) while (carrageenan treated animals= 17.6±2.0)

The values within parenthesis represents percent reduction in leukocyte migration after induction with carrageenan. Data represents Mean ± S.E.M (n=6) at *p<0.05, **p<0.01, ***p<0.005 as compared to control.

MPO enzyme activity which was significantly elevated at 4 hour of carrageenan administration. The methanol and ethyl acetate extracts (10-100 mg/kg) dose dependently inhibited (40-80%) the MPO levels in lung tissue as depicted in Table 1. Likewise, dexamethasone (0.5 mg/kg) also caused 58% reduction in the enzyme levels. It is well established that this corticosteroid is used clinically to treat many different inflammatory conditions such as allergic disorders, skin conditions, ulcerative colitis,

arthritis, lupus, psoriasis, or breathing disorders (Pithadia *et al.*, 2011). Based on these result, extracts of *A. corniculatum* inhibited the neutrophils influx which is also linked with the attenuation of adhesion molecules CD11b and CD18 (Frode *et al.*, 2001).

Moreover, the nitrate/nitrite levels in the pleural exudate were also estimated by Griess reaction (Dore *et al.*, 2007) in which both methanol and ethyl acetate extracts of *A. corniculatum* caused dose dependent

decline in exudate formation accompanied by the reduction in the levels of nitrate/nitrite ~80% at 100 mg/kg. (Table 1). Similarly, rutin (10 mg/kg) a quercetin-3-O-rutinoside also reduced the nitrate/nitrite levels by 77% because of its strong antioxidant properties. The NO levels significantly increased in the exudate of carrageenan-induced untreated group and plays an important role in mediating inflammatory responses a variety of sources that increased

the level of NO in lungs like expression of iNOs and stimulated neutrophils (Cuzzocrea *et al.*, 2000). Oxidative stress also plays an important role in inflammation because it activates the neutrophils due to which it activates ROS. NO is synthesized by iNOs it is a free radical and enhanced inflammation and interact with ROS to increase the activity of free radicals. As a consequence different pro-inflammatory cytokines IL-1 β , TNF- α are released that

Table 1: Effect of *A. corniculatum* Extracts on Myeloperoxidase and Nitrate/Nitrite Production

Treatment	Dose (mg/kg)	Myeloperoxidase (mU /ml)	Nitrate/Nitrite (μ M)
Saline (0.95% NaCl)		43 \pm 12.8	
Standard control	0.5 (Dexamethasone)	196 \pm 36.8 (58%)	–
	10 (Rutin)	–	20 \pm 4.0 (77%)
Carrageenan (1%)	0.1 ml	464.5 \pm 98.8	86 \pm 21.6
Methanol extract	10	220 \pm 21.6* (53%)	56 \pm 4.4 (35%)
	50	113 \pm 26.2** (76%)	34 \pm 4.3** (61%)
	100	80 \pm 8.1*** (83%)	20 \pm 4.0*** (77%)
Ethyl acetate extract	10	253 \pm 36.8* (47%)	28 \pm 2.1* (68%)
	50	200 \pm 40.8** (57%)	15 \pm 4.0** (83%)
	100	80 \pm 8.16*** (83%)	7.3 \pm 2.0*** (92%)

Data represents mean \pm S.E.M (n=6) of myeloperoxidase and nitrate/nitrite levels after treatment with carrageenan (1% ,0.1 ml) alone or in the presence of different concentrations of standards (dexamethasone and rutin), methanol and ethylacetate extracts.

The values within parenthesis represents percent reduction as compared to corresponding controls.

The significant values are shown with asterisks *p<0.05, **p<0.01, ***p<0.005.

activates the transcription factor NF- κ B (Elena *et al.*, 2012). Considering, the aforementioned mechanism of nitric oxide and its pro-inflammatory derivatives nitrates/nitrites in regulation of acute and chronic inflammatory process, inhibitory effects of *A. corniculatum* against MPO and nitrate/nitrite clearly demonstrates its potential against anti-inflammatory response.

The present investigation validated the traditional use of *A. corniculatum* against inflammation and rheumatism. Our data confirms the inhibitory effect of *A. corniculatum* upon both neutrophils and mononuclear infiltration in pleural cavity against carrageenan challenge in association with a parallel decline of nitrate/nitrite concentrations and MPO enzyme levels. Thereby, revealing a new protective mechanism of *A. corniculatum* extracts against acute lung inflammation.

Author's Contribution

Sabahat Aziz has performed the bench work and manuscript writing.

*Talat Roome has supervised the entire work and designed the experimental model.

Perbhat Ali and Anam Razzak performed experimental work and participated in literature surveys.

Muhammad Iqbal Choudhary supervised the chemistry part of the project.

Conflict of Interest

The authors affirm that there is no conflict of interest in this study and publication of this manuscript.

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Mini Review: Herbal Medicinal Products Regulation in Pakistan

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Abstract

Pakistan is gifted with a rich prosperity of curative plants from All Mighty Allah. Herbs have always been most important form of medicine in Pakistani population and currently plants have got popularity throughout the globe. Standardization, quality control and import and export of raw materials and herbal formulations materialize the key dispute for herbal drug manufacturers which is due to the non availability of sufficient regulatory strategy, principally for good manufacturing practice (GMP), no execution of good agricultural and collection practices (GACP), and weak implementation of the Herbal product drug act from the ministry of health of Pakistan. Now there are several opportunities for the development of quality of herbal products on the basis of data based studies which is essential for the establishment of the herbal products quality, safety and efficacy in the domestic and export market. Finally, strong Regulatory implementation becomes now necessary to moderate the hindrance for the commercialization of the herbal medicinal drug in Pakistan with the help of scientific research at Institutions, Universities and hi-tech advancement in the field of herbal medicine and their related products.

Keywords:

Herbal medicinal drugs, Quality control, Standardization, Regulatory requirement, Global scenario.

1. INTRODUCTION

Herbal medicines products are the manufacture of remedial experiences, by the generations of local practitioners of native systems of medicine as a dietary supplements derived from food plant, phytochemicals and pro-vitamins that now being described as functional foods, nutraceuticals and nutraceuticals or health foods etc. may support in maintaining good health and combating the different disease. Herbal medication are also in great insist in the developed world for primary health care due to their effectiveness, safety and fewer side effects, as well as providing treatment for those disorders which are associated with age such as memory loss, osteoporosis, arthritis and immune disorders, etc. due to the unavailability of modern medicine and only palliative treatment is processed. This is lead to unexpected enlargement in the herbal drug manufacturing and number of countries in spite of its prosperous traditional knowledge, customs of herbal medicines and large biodiversity only has a large share of the world market due to export and import of crude extracts and herbal drug products (Robinson and Zhang, 2011; WHO Geneva 2005). In these circumstance consumers, professionals and regulatory officials have struggled to change this scenario by gathering information about the quality, efficacy and safety of these products, and developed new guidelines for their registration in the regulatory framework as those

in the place of conventional pharmaceuticals according to their habitat and their distribution in the different localities.

1.1. World Paradigm of Herbal Medicine

Traditional medicines are categorized in many ways throughout the various jurisdictions around the world; a few of these are shown in Fig. 1. World Health Organization (WHO) published a report with name of "Traditional medicine strategy 2002-2005", which support the concept of a national policy on Traditional medicine/ Complementary and alternative medicine (TM/CAM). It has been exposed from that report, only 32% WHO member countries had a national policy. Response of another survey, based on whether laws or regulations existed on TM/CAM, indicated that 65% countries have law or regulations on herbal medicine (WHO, 2002-2005).

1.2. Alternative Medicinal Trend in Pakistan

In Pakistan, 80% country's population lives in rural areas where they followed alternative system of medicines as a first line of treatment. In 2009, a group of Government officials conducted the survey regarding the trend of utilization of medicine in different health care systems. Thus the assessment showed in Fig. 2 that revealed the trend of alternative therapies in Pakistan.

From the graph we can easily revealed that almost half of the population used TCAM which is to be a sign of an increasing popularity of CAM in Pakistan and report also showed the common practice of combined use of biomedicine with TCAM (Shaikh *et al.*, 2009).

1.3. Dosage Forms of Herbal Medicinal Products

Europe union definition of herbal medicinal

product is "Herbal medicine product is containing the active ingredients from plant origin or/and vegetative drug preparations". They are available in the many dosage forms as shown in Scheme 1. Dry extract available in pills/capsules/lozenges/ lyophilized powders in the market that was prepared by local Hakims or well known companies and dawakhanas (Shaista and Shahid, 2011) .

1.4. Growth of Herbal Medicinal Product Market

The modern annual sales trend of herbal medicines, from nine countries including Pakistan showed in Fig.3. The global market share or trade of traditional medicine was about 2500 species internationally and estimated at US\$83 billion annually in 2008, with an increase rate of sale per year and estimates suggest that it will reach up to the US\$ 5 trillion by 2050 (Kamboj, 2000; Hoareau and DaSilva, 1999; Robinson and Zhang, 2011).

12% of growing flora of Pakistan has been used in medicines and more than 300 medicinal plants are traded. In 1990s, 2 million kg of 200 medicinal plants were annually consumed by 10 principal Dawakhanas (Herbal manufacturers) of Pakistan that was bigger in the last two decades. According to calculated trade of medicinal plants approximately, 22 species of medicinal plants worth Rs.14.733 million while in 2002, this value eight-and-a-half times increased to more than Rs.122 million. Whereas consumption of 95 species of medicinal plants in 1990, valued Rs. 36 million while in 2002, valued Rs. 218 million which indicates the 6-fold increased in 12 years (Shinwari *et al.*, 2002). The 2012 market value of herbal products in contrast with the conventional medicine is 12% and now it is up to 16%.

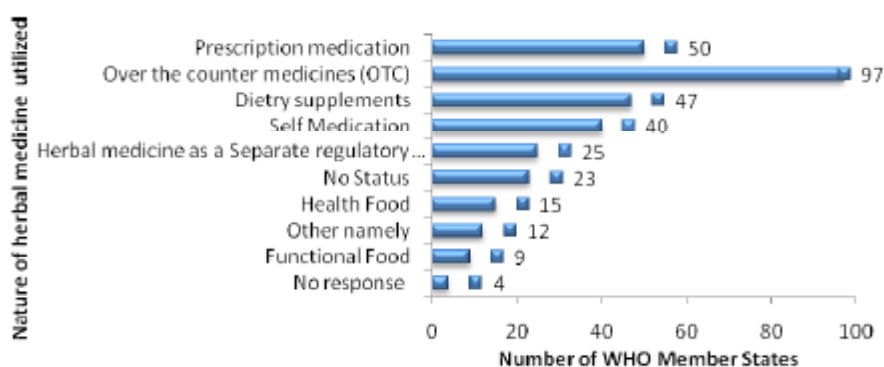
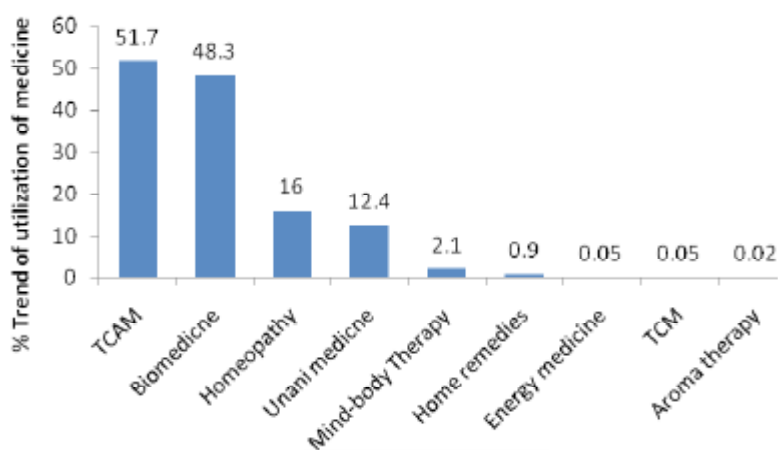
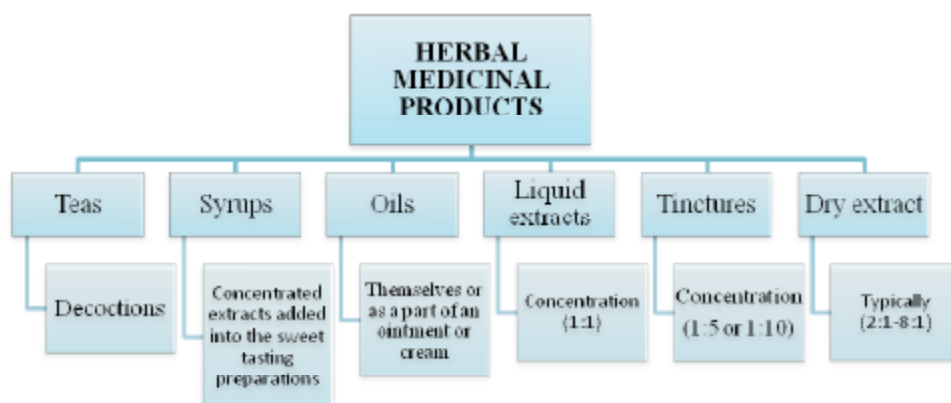


Fig. 1: Categories of regulation of herbal medicine in the world (WHO, 2002-2005)



TCAM: Traditional complementary alternative medicine
 TCM: Traditional complementary medicine

Fig. 2: Trends of alternatives therapies in Pakistan (Shaikh *et al.*, 2009)



Scheme 3: Different available dosage forms of herbal medicinal products

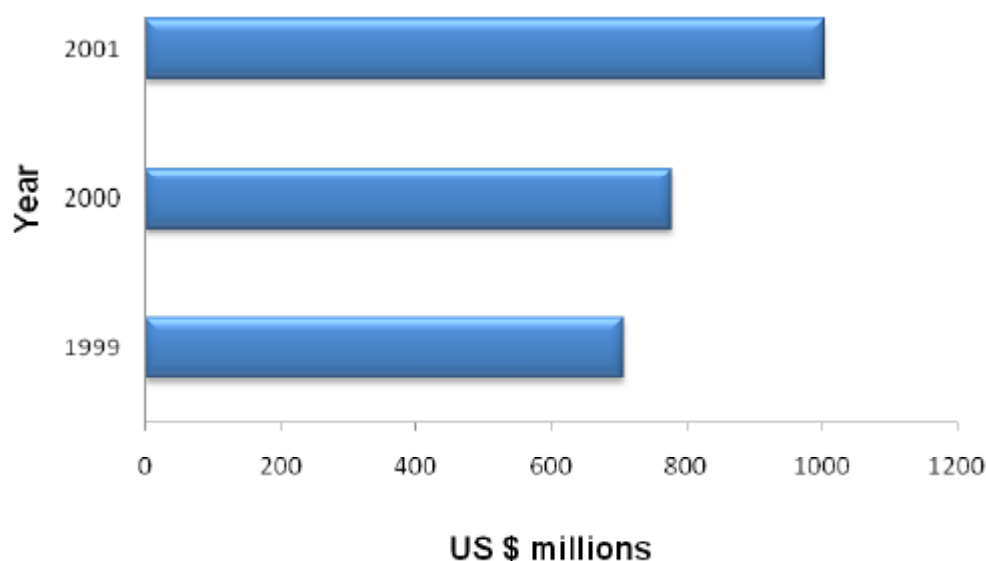


Fig. 3: Annual market sales of herbal medicines

1.5. Policy Concern in Relation to Standardization and Regulation Globally

From most recent decades there are various policies available in the national and international level for the regulation of alternative medicines. But the herbal drug and their products globally face the a variety of challenges like identification, recognition, consistent quality, learning, confirmation based research, safety and efficacy, coherent use, herb-drug relations, poor consideration of socio-cultural background of their practice and handling, security of patent rights of knowledge container, guarantee of natural supply, guideline and facility of non-formal practitioners, appropriate methodologies for evaluation and resolving conflicts. These all factors can be erasing by the linkage of raw material and finished herbal product in terms of quality control, standardization and regulatory

requirements. WHO published the various technical guide lines regarding the above mentioned issues such as:

- a) Good agricultural and collection practices (GACP)
- b) Good manufacturing practice for the herbal medicine (GMP)
- c) Guideline for the selection of the material for the quality control of herbal medicine in 2004 and 2005
- d) Monograph for various herbal drugs
- e) Guidelines for the assessment of herbal drug and quality control method of medicinal plant material

Through the utilization of above mentioned guidelines we can meet the increased demand of quality herbal medicinal products.

1.6. Herbal Drug Regulatory Status in Pakistan

In Pakistan, Traditional/Complementary medicines are being practiced under folklore, Unani, Ayurvedic and Homoeopathic (UAH) Act of 1965. In the year 2003 Government cabinet was approved amendments in the Unani, Ayurvedic and Homeopathic Practitioners Act, 1965 as the Tibb-e-Unani/Eastern Medicine Practitioners Act 1965 with a vision of officially registering Bachelors of Eastern Medicine and Surgery (BEMS) to practice Tibb. The Drugs Control and Traditional Medicines Division of the National Institute of Health Islamabad serve as the national institute on traditional medicine and were established in 1991. Moreover, Ministry of Health of Pakistan was established the expert committee on TM/CAM in 2001 for the development of national policy on Traditional medicines. The Drugs Act of 1962 only considers the regulation of herbal medicines advertising and prevention of its misuse. Later the bill had been prepared in 2010 for the regulation of manufacturing, storage, imports and export of Tibb-e-Unani, Ayurvedic, Homoeopathic, Herbal and Non-allopathic medicines (Shista and Shahid 2011).

Currently Drug Regulatory Authority of Pakistan directives to regulate herbal medicines according to the laws designed for allopathic medicines and they provide for effective coordination and enforcement of the Drugs Act, 1976 (XXXI of 1976) that will bring synchronization in inter-provincial buying and selling commercialization of therapeutic goods including herbal products as well (DRAP, Act, 2012).

As an agricultural country Pakistan still need some strong policies to furnish this law at the ground level that is from agricultural to the consumer hand.

2. FUTURE PROSPECTIVE

The importance of medicinal plants cannot be diminished in any society of the world due to its ancient utilization. As we know that the population of the world increases in both developed and developing world through the migration and birth rate and its gives the rising interest in the industrialized nations to have greatly expanded the claim of medicinal plants themselves and their prepared products. Therefore, in this scenario the regulatory authorities of the state have play a crucial role, in terms of value of herbal medicines, financing and appreciating training and research in the development of this field. For this instance, inclusion of some introductory modules of herbal medicinal knowledge into the basic medical curriculum of allopathic medical schools must be considered. Finally academicians, researchers, law & order regulators, herbal drug producer, and prescribers must join their hands together and could bring a new perspective of outstanding revolution by reorganization and redesign the herbal drugs. When all above concerns are given their valuable inputs as equivalent, successful, economical and safe partners then it can be possible to face the challenges of the 21st century for herbal drug.

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Obituary

Prof. Dr. M. Ataur Rahman
(1929-2015)

Inna lillahi wa inna ilayhi raji'un

“We surely belong to Allah and to Him we shall return

Al-Baqarah, II:156”.

Hamdard Medicus is sad to report the demise of Prof. Dr. Mohammad Ataur Rahman (D. Sc) an eminent scientist and one of the pioneer members of its Advisory Board. He breathed his last in Karachi on Saturday, 26th September, 2015. May Allah bless the departed soul with eternal peace (*Ameen*).

He also served as Professor of Bio-chemistry and Dean of Faculty of Science, Hamdard University, Karachi.

His contributions brought him many national and international honours and awards, including President of Pakistan's Award “Pride of Performance” and “Hilal-i-Imtiaz” by the Government of Pakistan. He received Gold Medal by Pakistan Academy of Sciences for outstanding contributions in research.

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