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Analysis of Phytochemicals in Petroleum Ether Leaves Extract from Abutilon Pannosum and Grewia Tenax by Liquid Chromatography/ Quadrupole Time-of-Flight Mass Spectrometry (LC/Q-TOF-MS) after Continuous Hot Percolation Successive Soxhlet Extraction

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Abstract: *Purpose of the present study was to carry out extraction, isolation and identification of phytochemical constituents present in leaves extracts of Abutilon pannosum and Grewia tenax collected from Kachchh region, Gujarat by use of Liquid Chromatography Quadrupole Time of Fight Mass Spectrometry (Q-TOF LC/MS). Successive extraction of plant leaves was undertaken by Soxhlet extractor using petroleum ether (35-60°C) as a solvent. Crude petroleum ether extracts were injected in Q-TOF LC/MS instrument for isolation and identification of useful phytochemicals. The result of phytochemical analyses showed that there are very important phytochemicals found in petroleum ether extracts of A. pannosum leaves like Alkaloids, fatty acids, sterol lipid, heterocyclic compound and G. tenax leaves have Fatty acid, alkaloid, flavonoid, terpenoid, sterol lipid and heterocyclic compound, cannabinoid, carotenoid etc and other medicinal activity. Thus, petroleum ether extract of G. tenax leaf gives good medicinal activity compared to the petroleum ether of A. pannosum leaf part. In this study, the G. tenax petroleum ether extract have greatest number of bioactive compounds.*

Keywords: *Abutilon pannosum, Grewia tenax, Q-TOF LC/MS, Phytochemicals, Petroleum ether extract, Soxhlet extraction*

1. INTRODUCTION

Diverse plants have been used in different parts of the world to treat of human diseases and infections. [1, 2, 3] Plants are used medicinally in different countries and are a source of different potential and powerful drugs. [4] The phytochemicals constituents are responsible for medicinal activity of plant species. The phytochemicals are group into two important classes. [5] Primary components which include amino acids, common sugars, proteins and chlorophyll etc. and secondary components consisting of alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic composites etc. [6] The qualitative analysis as well as quantification of phytochemical constituents of a medicinal plant is considered to be a vital step in any kind of medicinal plant research. [6]

1.1. Abutilon Pannosum

Abutilon is one of the fundamental genuses of malvaceae family, commonly known as khapat. [7] Various species of the genus *Abutilon* is used in local medicines for the treatment of various ailments. Among this, *Abutilon pannosum*, is an under shrub and is distributed in India, Pakistan, Tropical Africa, China and Arabia. [8] We were focused in kachchh district region species. The leaves of *A. pannosum* were used as adjunct to medicines used for stack complaints. The plant contains mucilage, tannins asparagines, Gallic acid and sequiterpens. Its extract is also used in relieving thirst, in treating bronchitis, diarrhea, gonorrhea and inflammation of the bladder and in reducing fever. In addition, it is used in cleaning wound and ulcer, treating vaginal infection, diabetics, hemorrhoids and can also use as an anemia. [9]

1.2. Grewia Tenax

Grewia tenax is one of the important genus of Tiliaceae family, commonly known as Gangeti a valuable plant species in kachchh**.** [10] The plant has high medicinal values and is widely used for the treatment of various common diseases. [11] Roots are used to treat against jaundice, pulmonary infections and asthma. [11] Leaves are used against trachoma, tonsillitis infections and are used as a poultice to treat swelling and an alcoholic extract ointment was reported to help in faster wound healing. [11] Fruits are small berries, round, orange sweetened and it may be consumed either fresh or dried. [12] The plant preparations are used for the treatment of bone fracture and for bone strengthening. [13]

2. MATERIALS AND METHODS

2.1. Collection of Plant Samples

This study was conducted in October 2016. Leaves of Abutilon pannosum and *Grewia tenax* were collected from farm of padmavati temple, Asambiya road and Punitvan Bhuj respectively.

2.2. Preparation of Samples

The fresh, healthy plant leaves of *Abutilon pannosum* and *Grewia tenax* were washed with water to remove dirt and foreign materials and properly dried in shade for 2-3 weeks. Finally, crunchy leaves were pulverized in a mortar grinder, sieved through mesh screen and stored in air tight bag.

2.3. Materials Required

Abutilon pannosum and *Grewia tenax* leaf powder, petroleum ether, RBF (round bottom flask), condenser, heating mantle, measuring flask and thimble.

2.4. Preparation of Extracts

15 gm of leaf powder was extracted with 2-3 litre of petroleum ether (60°- 64°c) 95 % using soxhlet apparatus by continuous hot percolation method. After extraction, it was filtered and the removal of solvent was done under pressure by distillation process. Extract were collected in air tight glass tube. The prepared extract dissolve in 0.9ml methanol and 0.1ml 0.1% formic acid in glass tubes, for detection of the investigated compounds was achieved using a quadrupole coupled to time-of-flight analyzer (Q-TOF-MS 6540, Agilent Technologies, UHD). The mass spectrometry was equipped with an ESI Jet Stream source; identification and determination of the investigated drug was carried out in the SCAN mode.

2.5. LC-Q-TOF-MS Method

The separation of the analysts was carried out using an Agilent LC-Q-TOF-MS 6540, UHD.

2.5.1. LC-Parameter

The injected sample volume was 10µL; Mobile phases A and B were water and acetonitrile with 0.1% formic acid, respectively. The flow rate was 0.6mL/min. A 16 min run time was used after each analysis. The optimized chromatographic method held the initial mobile phase composition (10% B) constant for 0 min, followed by a linear gradient to 100% B after 14 min. and return back (10% B) at 14 min. the system featured a binary pump and vacuum degasser, well-plate auto sampler with a six-port micro-switching valve, a thermo stated column compartment. Samples were loaded onto a Reprosil C18 column (2.0mm×150mm, 2.5 µm – Dr Maisch, Germany) for metabolite separation.

2.5.2. Q-TOF Parameter

The LC system was connected to an Agilent 6450 ultra-high definition quadrupole time-of-flight mass spectrometer equipped with dual electro spray Jet Stream Technology operating in positive ion mode. The operating parameters were as follows: capillary voltage: 4000V; nebulizer pressure: 45 psi(N_2); drying gas: 8 L/min; gas temperature:325°C; nozzle voltage: 1000V; fragment or voltage: 150V; skimmer voltage: 65V, m/z; 100 to 1700, sheath gas temp350 °C and sheath gas flow 11 L min^{-1}. The data recorded was processed with Agilent Mass Hunter software. Accurate mass measurements of each peak from the total ion chromatograms were obtained by means of an automated calibrant delivery system using a low flow of a calibrating solution (Calibrant solution A, Agilent Technologies, Santa Clara, CA, USA).

3. RESULT

3.1. A. pannosum Petroleum Ether Extract

Table I represents petroleum ether extract of A. pannosum. It gives fatty acid, alkaloid, flavonoid, sterol lipid and heterocyclic compound.

3.2. G. tenax Petroleum Ether Extract

Table II represents petroleum ether extract of *G. tenax*. It gives Fatty acid, alkaloid, flavonoid, terpenoid, sterol lipid and heterocyclic compound, cannabinoid, carotenoid, and also illustrate anticancer, antibiotic for veterinary use, anti-hemorrhagic, prothrombogenic, antibacterial activity, antimicrobial, antioxidant, anti-tumor, anti-emetic, anti-hyperalgesic, local anaesthetic effect intraderm, anti-inflammatory, antidiabetic activity, antidepressant activity, antihistamine and anticholinergic, fungicide, anticholinesterase, antitumor, and for asthma, Prevent allergic reactions, As conduct of certain types of autoimmune diseases, and other lung conditions, skin conditions, leukemia, multiple myeloma lymphoma, and asthma. It is similarly useful in vomiting and nausea, prevention and treatment of graft-versus-host disease following allogeneic bone marrow transplantation, in rubber synthesis, acaricidal and insecticidal, preparation of cosmetic and dermatological compositions. *G. tenax* gives highly medicinal value in petroleum ether extract its data is shown in below.

4. DISCUSSION

The secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value.[106] For example Flavonoids are phenolic compound and plant phenolics are a main group of compounds that act as antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity, [110] it have been exposed to display their actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A2.[111] Flavonoids assist as health promoting composite as a results of its anion radicals. [112] These notes sustenance the usefulness of this plant in folklore medicines in the treatment of stress connected diseases and as coverings for wounds usually encountered in circumcision rites, bruises, cuts and sores. [113, 114] Flavonoid, fatty acid, alkaloid, sterol lipid and heterocyclic compound were present in both plant leaf sample of petroleum ether extract. Cannabinoid, Carotenoid etc were present in only *G. tenax* leaf sample of petroleum ether extract. Cannabinoids were only present in *G. tenax* plant of petroleum ether extract. Cannabinoids are pharmacological agents which alleviate pain through a variety of receptor and non-receptor mechanisms including direct analgesic and anti-inflammatory effects, modulatory actions on neurotransmitters, and interactions with endogenous and administered opioids. Cannabinoid agents are currently available in various countries for pain treatment, and even cannabinoids of botanical origin may be approvable by FDA, although this is distinctly unlikely for smoked cannabis. [115, 116] Carotenoids may act as antioxidants and may exhibit chemo preventive anti atherosclerotic effects and anticancer effects. [117] The above result also showed that *G. tenax* has anticancer, antibiotic for veterinary use, anti-hemorrhagic, prothrombogenic, antibacterial activity, antimicrobial, antioxidant, anti-tumor, anti-emetic, anti-hyperalgesic, local anaesthetic effect intraderm, anti-inflammatory, antidiabetic activity, antidepressant activity, antihistamine and anticholinergic, fungicide, anticholinesterase, antitumor, and for asthma, Prevent allergic reactions, As usage of certain kinds of skin conditions, other lung conditions, autoimmune diseases, and asthma leukemia, multiple myeloma, and lymphoma etc. So it can be derived that the *G. tenax* petroleum ether extract has the maximum number of bioactive compounds.

5. CONCLUSION

The present study was prepared on extraction isolation and identification of phytochemical from leaf petroleum ether extract of *A. pannosum* and *G. tenax* by using LC-Q-TOF-MS. The presence of phytoconstituents make the plant useful for treating different ailments and have a potential of given that helpful drugs of human as well as veterinary use. In the present study, we have found that most of the biologically active phytochemicals were present in the petroleum ether extracts of *G. tenax* compare to *A. pannosum* of leaf part. Thus, *G. tenax* give good medicinal activity compared to the *A.*

pannosum. Since the petroleum ether extract of leaf contains more constituents, it can be considered beneficial for further investigation. The phytochemical investigation of that medicinal plants are also significant and have profitable attention in both pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases and research institutes. Thus it can be predictable that the vital phytochemical properties recognized by this study on native plant of Kachchh region will be useful against diverse diseases.

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