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Research Paper

Anti-Oxidant Activity and Total Phenolic Content (TPC) Of the Plant Extract Of Abutilon *Indicum* (Linn).

S.KRISHNAMOORTHY* & K.KAMATCHI
Assistant Professor, PG and Research Department of Zoology, Vivekananda College, Tiruvedakam West Madurai, Tamil Nadu, India

ABSTRACT:
The present study deals with analyse the antioxidant activity and total phenolic content of A. indicum extract by using different solvents such as aqueous, actone and ethanol extracts were prepared to study the total phenolic, flavonoid and tannin content. All these chemicals were not extractable in one solvent. In A. indicum, Alkaloid, steroid, terpenoid and phlobatannins only present in Aqueous extract In Acetone extracts tannin, flavonoid, steroid, Alkaloid and quinine were present rest of all the components were absent. For ethanol, Tannins, Phlobatannins, Flavonoids, Steroids, Quinone, Coumarin, Terpenoids and Glycosides were present; Not a single solvent showing a complete result. The maximum phytochemicals was found in ethanolic extract of experimental plant. The dose response curve of DPPH radical scavenging activity of crude extracts of plant was observed, Antioxidant activity in the form of IC50 values of different extracts were calculated. Highest antioxidant activity was given by A. indicum extract at the concentration of 170µg/ml among all the ethanolic leafs which is found to be more than standard. The FT-IR results interprets that, so many aromatic, aliphatic, ring compounds were present in the experimental samples.

KEYWORDS: *Abutilon indicum, Antioxidant activity, Total Phenolic Content*

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I. INTRODUCTION:

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1].

Extraction (as the term is pharmaceutically used) is the separation of medicinally active portions of plant (and animal) tissues using selective solvents through standard procedures. The products so obtained from plants are relatively complex mixtures of metabolites, in liquid or semisolid state or (after removing the solvent) in dry powder form, and are intended for oral or external use. These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts or powdered extracts. Such preparations have been popularly called galenicals, named after Galen, the second century Greek physician [2].

Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity [1].

The purpose of standardized extraction procedures for crude drugs (medicinal plant parts) is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent known as menstrum. The extract thus obtained, after standardization, may be used as medicinal agent as such in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsules. These products contains complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignans [3].

The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction, and phytonic extraction (with hydrofluorocarbon solvents). For aromatic plants, hydrodistillation techniques (water distillation, steam distillation, water and steam distillation), hydrolytic maceration followed

by distillation, expression and enfl eurage (cold fat extraction) may be employed. Some of the latest extraction methods for aromatic plants include headspace trapping, solid phase micro-extraction, protoplast extraction, microdistillation, thermomicrodistillation and molecular distillation [3].

Plant material

Plants are potent biochemists and have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc i.e. any part of the plant may contain active components. The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories. Scientific analysis of plant components follows a logical pathway. Plants are collected either randomly or by following leads supplied by local healers in geographical areas where the plants are found [1, 4,5].

Choice of solvents

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions includes, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate. The factors affecting the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the extractants [6]. The choice of solvent is influenced by what is intended with the extract. Since the end product will contain traces of residual solvent, the solvent should be non-toxic and should not interfere with the bioassay. The choice will also depend on the targeted compounds to be extracted [1, 4].

Free radicals are chemical species which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Free radicals are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function [7] .

Ultraviolet light, ionizing radiation, chemical reactions and metabolic processes can induce the production of reactive oxygen species (ROS) in the cells. Free radicals can initiate the oxidation of bio molecules, such as protein, lipid, amino acids and DNA which will lead to cell injury and can induce numerous diseases [8].

Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases, including cancer, cardiovascular diseases and inflammatory diseases. This activity is due to the ability of antioxidants to reduce oxidative stress by neutralizing or scavenging of reactive species by hydrogen donation [9].

Medicinal plants contain some organic compounds which produce definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [10,11].

Phenolics are especially common in leaves, flowering tissues and woody parts, such as stems and barks. Studies have shown that they play an important preventive role in the development of cancer, heart diseases and ageing related diseases [12].

Abutilon indicum (Linn) family (Malvaceae) commonly known as 'Thuthi /Atibala' is distributed throughout the hotter parts of India [13] and used in our Traditional System of Medicine for healing various diseases. Almost all parts of Atibala are of medicinal importance and are used traditionally for the treatment of various ailments. The roots of the plant are considered as demulcent, diuretic, used in chest infection and in urethritis. The infusion of root is described in fevers as cooling medicines and is useful in stangury, haematuria and in leprosy. The leaves are effective in ulcer, for the treatment of diabetes, diuretic infection and gingivitis. Fomentation of plant materials are used to relief body pain. The decoction of the leaf is used in toothache, tender gums and internally for inflammation of bladder. In some places, juice from the leaves in combination with the liquid extract of *Allium cepa* is used to treat jaundice, and in cases of hepatic disorders [14,15].

The bark is used as febrifuge, anthelmintics, alexeteric, astringent and diuretics. The seed are used in piles, laxative, aphrodisiac, expectorant, in chronic cystitis, gleets and gonorrhea [16,17,18,19]. The leaves and seeds are crushed with water to form paste which is applied to penis to cure syphilis. [20, 21, 22].

In Siddha system of medicine, it used as a remedy for jaundice, piles, ulcer and leprosy [23] . It also exhibits marked hepatoprotective action, which has been related to their antioxidant properties. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability [24].

Extensive review on the effect of free radicals and antioxidants in normal physiological functions and human were studied [25] . It is possible to reduce the risks of chronic diseases and prevent disease progression

by either enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants [26] .

Alkaloids, flavonoids, steroids, terpenoids and saponins have been isolated and characterized from genus Abutilon [27, 28]. Previous phytochemical investigations of Abutilon indicum showed the presence of seven flavonoids, two sesquiterpene lactones, [29] gallic acid, β -sitosterol, geraniol and caryophylline [28] . The analysis of phenolic compounds in plants is of considerable commercial importance, since it is known that they contribute to the flavor and antioxidant property [30] . However, no attention has been paid to its comparative Total Phenolic Content (TPC) and antioxidant status of stem extracts. Thus a common spectrophotometric method for total polyphenol content according to Follin-Ciocalteu has been widely used in the area of oncology and viticulture [31] and in-vitro antioxidant property by common DPPH Scavenging method. In the present investigation these in-vitro studies were carried out for the first time to study the comparative properties of total polyphenol content (TPC) and its antioxidant properties on stem extracts.

In the present investigation these in-vitro studies were carried out for the first time to study the comparative properties of total polyphenol content (TPC), FT-IR analysis and its antioxidant properties on stem extracts.

II. MATERIALS AND METHODS

Plant description:

Found in hilly area upto 1,200 m and in hotter parts of rajasthan. Medium sized, branched perennial shrub. Upto 2 meters in height. Plant covered with minute hairs. Leaves are alternate, cordate and acute. Flowers are yellowish, with 5 petals. Fruits have 15-20 chambers, arranged spirally. Seed color is blackish brown. This plant is useful in gout, tuberculosis, ulcers, bleeding disorders, and worms. It cures burning sensation. Decoction used in toothache and tender gums. Leaves are locally applied to boils and ulcers. Roots are used in fever, chest affection and urethrities.

Preliminary Phytochemical Screening

The ethanolic extracts of following plants was subjected to different chemical tests for the detection of different phytoconstituents using standard procedures.

Test for Tannins: 1 ml of the sample was taken in a test tube and then 1 ml of 0.008M Potassium ferricyanide was added. 1 ml of 0.02 M Ferric chloride containing 0.1 N HCl was added and observed for blue-black coloration.

Test for Phlobatannins: When crude extract of each plant sample was boiled with 2 % aqueous HCl. The deposition of a red precipitate was taken as evidence for the presence of phlobatannins.

Test for Saponins: Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. Add some drops of olive oil. The formation of stable foam was taken as an indication for the presence of saponins.

Test for Flavonoids: 5 ml of dilute ammonia solution were added to a portion of the crude extract followed by addition of concentrated H2SO4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

Test for Steroids: 2 ml of acetic anhydride was added to 0.5 ml crude extract of plant sample with 2 ml H2S04. The colour changed from violet to blue or green in samples indicates the presence of steroids.

Test for Alkaloids: Crude extract was mixed with 2 ml of Wagner's reagent. Reddish brown colored precipitate indicates the presence of alkaloids.

Test for Quinones: Dilute NaOH was added to the 1 ml of crude extract. Blue green or red coloration indicates the presence of quinones.

Test for Coumarin: 10 % NaOH was added to the extract and chloroform was added for observation of yellow color, which shows the presence of Coumarin.

Test for Terpenoids (Salkowski test): 5 ml of extract was mixed with 2 ml of chloroform and 3 ml of concentrated H_2S0_4 was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for Cardiac glycosides (Keller-Kiliani test): 5 ml of extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated H_2SO_4. A brown ring of the interface indicates a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Quantitative Determination of Phytochemical Constituents Determination of TPC

Total phenolic content of the methanolic extract of *A. indicum* plant was determined by standard method [33] with little modifications, using tannic acid as a standard phenolic compound. The extracts were diluted with distilled water to a known concentration in order to obtain the readings within the standard curve

range of 0.0 to 600 µg of tannic acid/ml. 250 µl of diluted extract or tannic acid solution was mixed with 1 ml of distilled water in a test tube followed by the addition of 250 µl of Folin - Ciocalteu reagent. The samples were mixed well and then allowed to for 5 min at room temperature in order to allow complete reaction with the Folin-Ciocalteu reagent. Then, 2.5 ml of 7 % sodium carbonate aqueous solution was added and the final volume was made up to 6 ml with distilled water. The absorbance of the resulting blue colour solution was measured at 760 nm using spectrophotometer after incubating the samples for 90 min. All the experiment was conducted in three replicates.

Determination of Tannin

5 g of the dried powder of each sample was weighed into a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added. The mixture is covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath until it reaches to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried, weighed and percentage was calculated [32, 34].

Determination of Flavonoids

10 g of each plant sample was extracted repeatedly with 100 ml of 80 % aqueous methanol at room temperature. The whole solution was filtered through Whattman filter paper No 41. The filtrate was allowed to be evaporated into dryness over a water bath and weighed to a constant weight [35].

***In vitro* Antioxidant activity**

1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH radical scavenging method was used to evaluate the antioxidant property. The antioxidant activity was compared with that of the natural antioxidant, ascorbic acid. The concentrations of the plant extracts required to scavenge DPPH showed a dose dependent response. The antioxidant activity of each sample was expressed in terms of IC50, and was calculated from the graph after plotting inhibition percentage against extract concentration DPPH assay was carried out after making some modifications in the standard protocol [15]. 1.5 ml of 0.1 mM DPPH solution was mixed with 1.5 ml of various concentrations (10 to 500 µg/ml) of leaf extract. The mixture was shaken vigorously and incubated at room temperature for 30 min in the dark. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm by a spectrophotometer. The solution without any extract and with DPPH and methanol was used as control. The experiment was replicated in three independent assays. Ascorbic acid was used as positive controls. Inhibition of DPPH free radical in percentage was calculated by the formula:

Inhibition (%) = [(Acontrol-Atest)/Acontrol] × 100

Where A control is the absorbance of the control (L-Ascorbic acid) and Atest is the absorbance of reaction mixture samples (in the presence of sample). All tests were run in triplicates (n=3), and average values were calculated.

IC50 value

Inhibition Concentration (IC50) parameter was used for the interpretation of the results from DPPH method. The discoloration of sample was plotted against the sample concentration in order to calculate the IC50 value. It is defined as the amount of sample necessary to decrease the absorbance of DPPH by 50 %.

FOURIER TRANSFORM INFRARED SPECTROPHOTOMETER (FTIR)

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined.

The samples were given for analysis in Madurai Kamaraj University. Dried powder of different solvent extracts of each plant materials were used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a Scan range from 400 to 4000 cm1 with a resolution of 4 cm^{1}

III. RESULTS AND DISCUSSION

Qualitative Analysis of Phytochemicals:

The phytochemical analysis of different chemical compounds (Tannins Phlobatannins Saponins Flavonoids Steroids Alkaloid Quinone Coumarin Terpenoids Glycosides) were tested in different extracts such as aqueous, actone and ethanol extracts respectively. However, all these chemicals were not extractable in one solvent. In *A. indicum*, Alkaloid, steroid, terpenoid and phlobatannins only present in Aqueous extract In Acetone extracts tannin, flavonoid, steroid, Alkaloid and quinine were present rest of all the components were absent. For ethanol, Tannins, Phlobatannins, Flavonoids, Steroids, Quinone, Coumarin, Terpenoids and Glycosides were present; Not a single solvent showing a complete result. The maximum phytochemicals was found in ethanolic extract of experimental plant.

A. indicum extract by using different solvents such as aqueous, actone and ethanol extracts were prepared to study the total phenolic, flavonoid and tannin content. The yield of extract obtained from 15gm of dry plant material was measured for each extract. The total phenolic content in the plant extracts was examined using Folin-Ciocalteus reagent and is expressed in terms of Gallic acid equivalent. The values obtained for the concentration of total phenolics are expressed as mg of GA/g of extract.

The total phenolic content in the examined extracts ranged from 2.2to 21.6 mg GA/g. The high concentrations of phenolics were obtained from ethanolic extract of *A. indicum*. The total flavonoid content in the examined extract ranged from 1.8to 23.2 mg QE/g. The highest concentration of flavonoid was measured in the ethanolic extract of *Cassia serecea*. The total tannin content in the examined extracts ranged from 1.2 to 4.5 mg GA/g. The highest concentration of Tannin was measured in the ethanolic extract of *A. indicum.*

DPPH activity:

DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts. DPPH radical scavenging activities of the extracts depended not only on plant type but also upon the extraction solvent. In general, DPPH scavenging activities increased with increasing phenolic components such as flavonoids, phenolic acids, and phenolic diterpenes. These phenolic components possess many hydroxyl groups including o-dihydroxy group which have very strong radical scavenging effect and antioxidant power.

In the DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The molecule of 1, 1-diphenyl-1, 2-picryl hydrazine is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecules a whole. The delocalisation also gives rise to the deepviolet colour, characterized by an absorption band in methanol solution centered at 517 nm. The dose response curve of DPPH radical scavenging activity of crude extracts of plant was observed, Antioxidant activity in the form of IC50 values of different extracts were calculated and shown in table 3. Highest antioxidant activity was given by *A. indicum* extract at the concentration of 170µg/ml among all the ethanolic leafs which is found to be more than standard. Thus it is clear that, polyphenolic antioxidants in leaves of selected plants play an important role as bioactive principles and the scavenging effect can be attributed to the presence of active phytoconstituents in them.

FT-IR Results:

So many aromatic, aliphatic, ring compounds were present in the experimental samples. The peak values are obtained from FT-IR results shows that 611.45 665.46 775.41– Aliphatic Iodo groups, 900.79 and 1045.45; 1105.25 – Aliphatic bromo compounds, 1247.99 - Alcohol hydroxyl compound rings, 1321.28 - Hydroxy groups, 1375.29 – dimethyl groups, 1427.37 – Saturated aliphatic groups like methylen C-H bends, 1512.1 – aromatic ring stretch, 1627.97 – Organic nitrates, 1730.21-Methyl groups; 2858.6, 2924.18, 3423.76-Ammonia groups were found the present experimental samples (Fig.1).

This concludes the summary data for a first-pass assessment of an infrared spectrum, based on the most common and characteristic group frequencies. The technique is not foolproof, but for many simple compounds, if one systematically applies the diagnostic tests on the spectrum from a sample, as presented above, it is possible to gain some understanding of the chemical functionality. It is always hoped that more information can be gained from the sample, either by first-hand knowledge or by asking questions, or by performing additional tests. Once a basic interpretation is accomplished, and the sample is broadly characterized, it is recommended that reference spectra are used to try to obtain a more exact match to the sample.

Adegboye *et al.,* (2008) reported that the medicinal values of medicinal plant lies in these phytochemical compounds and as such procedure a definite physiological action on the human body. Saxena, (1989) also reported these phytochemical compounds were bioactive, easily that biodegradable and of narrow-spectrum activity against plant diseases. The anti-inflammatory effects of alkaloids and alkaloids and flavonoids was reported by (Hodek *et al.,* 2002). The effectiveness of glycosides in the treatment of congestive heart failure

was reported by (Yakari *et al.,* 1995); while tannins and steroids were found to be used in the treatment of inflamed or ulcerated tissues.

Medicinal value of plants have assumes an important dimension in the past few decades. Plants produce a very diverse group of secondary metabolites with antioxidant potential. Antioxidants block the action of free radicals which have been implicated in the pathogenesis of many diseases and in the aging process [36,37,38]. An important role is being played by free radicals in governing the various biological processes which are necessary for the body. They have their role in implicating cell-signaling mechanism occurring in our body. This shows that free radicals are necessary but at the same time harmful for the body. Hence it has a number of mechanisms to minimize free radical induced damage. The damage was repaired with the help of several enzymes like superoxide dismutase, catalase, glutathione, peroxidase and glutathione reductase. In addition antioxidants play a key role in these defense mechanisms which are normally vitamin A, vitamin C, vitamin E and polyphenols [39]. In a study, chemical composition and some anti-oxidant indices of *Alstonia boonei* stem bark extract were evaluated. Presence of alkaloids, tannins, saponins, flavonoids and cardiac glycosides were detected together with important vitamin, ascorbic acid. DPPH radical scavenging activity, total phenolic content and reducing power were 41.58 %, 2.09 mg/g gallic acid equivalent and 0.32 respectively.

Results of work had indicated that phytochemicals were responsible for medicinal effects of this plant [40]. In another study, phytochemical constituents and medicinal properties of different extracts of *Anacardium occidentale* and *Psidium guajava* was studied [41]. Aqueous and methanol extracts of leaf, bark, and root cashew and guava were analysed quantitatively for tannin, total polyphenol, oxalate, saponin and alkaloids. Highest concentrations of the bioactive principles were detected in ethanolic extracts of the plants except in the case of saponin where hot water extract produced the bioactive principle. In guava was found tannin-11.5 mg/g, total polyphenol-1.67 mg/g, alkaloid-59.85 % and oxalate-6.66. In cashew tannin-15.38 mg/g, total polyphenolics-2.0 mg/g, alkaloid-39.9 % and oxalate-8.13 %

was detected. The study had proved that the presence of these phytochemicals enhances the efficacy and dilutes toxicity also. The total phenolic content of selected Jordanian plant species was done and established that antioxidant activity was closely correlated with phenolic content [42].

During the present work, it was found that *Abutilon indicum* exhibited higher antioxidant activity with higher phenolic content. So these findings are in agreement with previous reports that there is linear relation between antioxidant activity and total phenolic contents. Therefore, it can be suggested that the phenolic compounds significantly contributed to the antioxidant potential of selected plant species. The results are also in agreement with another study done on 112 traditional Chinese medicinal plants [43].

Other group had performed phytochemical analysis of 13 medicinally important plants of Margalla hills and surroundings [44] and investigated the qualitative and quantitative analysis of the major bioactive constituents. Alkaloids, saponins, tannins, anthraquinones, flavonoids, flavons, flavonols and chalcones, terpenoids, phlobatanins, coumarins, steroids and cardiac glycosoides were analyzed qualitatively whereas alkaloids, flavonoids, tannins, phenols and saponins were analysed quantitatively too. Quantitative analysis of total polyphenols, tannins, proanthocyanidins and flavonoids in 20 Serbian and Chinese cultivars of *Soybean* (*Glycine max* L.) was performed [45].

Phytochemical and nutrient evaluation of *Spondias mombin* leaves was performed and reported the qualitative and quantitative analysis of various groups of chemical constituents, minerals and vitamins [46]. The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit. Quantitative phytochemical estimation and antioxidant studies on aerial parts of *Naravelia zeylanica* DC was done [47]. It was a woody climber belonging to Ranunculaceae family and whole plant is used as medicine for different problems. Powdered plant material was found to have alkaloid 0.86%w/w, total phenol 0.72 %w/w, tannin 8.72 %w/w, flavonoids 0.56 %w/w and saponin 2.86 %w/w were present in the aerial parts. High concentration of phenols and tannins in this plant cause greater reducing powered which in turn responsible due to the presence of these constituents. Quantitative estimation of methanolic extract of various phytoconstituents *viz* total tannins (156.5 mg/g), total phenolics (146.40 mg/g), total flavonoids (30 mg/g) and total flavonols (3.6 mg/g) content on *Cinnamomum wightii* Meissn flowers of family Lauraceae which may serve as diagnostic tools for identification of crude drug [48].

Qualitative and quantitative analysis on plants like *Gymnema sylvestre, Tinospora cordifolia, Lawsonia inermis, Azadirachta indica* and *Ocimum sanctum* having same ayurvedic properties [49-53].

Phytochemical studies on methanolic and ethyl acetate extracts of leaves of *Anogeissus leiocarpus* and showed that the plant contains alkaloids (152.0 ± 0.1 mg/g), phenolics (1294.81 ± 3.0 mg/g), flavonoids (330.7 ± 3.0 mg/g) in the methanol extra ct and alkaloids (80.20 ± 0.0 mg/g), phenolics (616.5 ± 4.4 mg/g), flavonoids (202.5 ± 4 .0 mg/g) in the ethyl acetate extract respectively The methanol extract of the leaves of the plant *Leucas indicum* has been tested for the determination of antioxidant activity by reducing assay and found that reducing power increases with the increase in concentration of the crude extract. Free radical scavenging potential of the different extracts of leaves of *Oroxylum indicum* (L.) Vent. (Bignoniaceae), one of the widely

used medicinal plant, was evaluated *in vitro* by using diphenylpicryl- hydrazyl (DPPH) assay. The results were expressed as IC50.

Ascorbic acid was used as standard showed an IC50 of 24.0 ug/mL, whereas, the crude ethyl acetate (I), methanolic (II) and water (III) extracts of leaves of *O. indicum* showed IC50 values of 49.0, 55.0 and 42.5 respectively at 100 mg/mL concentration. Antioxidant activity evaluation of 28 Chinese herbs was performed by DPPH and reducing power assays in which *Artemisia vulgaris* and *Sanguisorba officinalis* showed the best antioxidant performance in both the tested methods . Reducing power assay of Petroleum ether extracts of *Eichhornia crassipes* (Mart.) Solms was studied at different concentrations and time delay and found to be increase with increase in concentration and time. The extracts were compared to standard antioxidant L-ascorbic acid. All the extracts showed greater reducing power than that of the standard .*In vitro* antioxidant testing of the extracts of *Samanea saman* (Jacq.) Merr in different solvents like petroleum ether, chloroform, ethyl acetate, aqueous and HCl was performed and found that mainly antioxidants were extracted in petroleum ether and had shown maximum reducing power[54-57] .

IV. CONCLUSION

Phytochemical screening of ethanolic extracts of *Abutilon indicum* had revealed the presence flavonoids, tannins, terpenoids, saponins, steroids, alkaloids by positive reaction with the respective test reagent. Results obtained in this investigation indicate that ethanolic extract of *A. indicum* , rich in phenolics exhibited highest antioxidant and reducing activities. Total phenolic content had positive correlation with antioxidant capacity. It was observed that the leaf extract contained high level of phenolic content that might have accounted for the strong activity observed against DPPH radicals. The finding of this study suggests that this plant leaves could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of ageing and age associated oxidative stress related degenerative diseases. Further investigation on the isolation and characterization of the antioxidant constituents is however required.

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Table 1. The qualitative test for phytochemical analysis of various extracts of *A. indicum*

	Solvents		
Compounds	**Ethanol**	**Acetone**	**Aqueous**
Tannins	+	++	-
Phlobatannins	++	-	++
Saponins	-	-	-
Flavonoids	++	+	-
Steroids	+	++	++
Alkaloid	-	+	+

Quinone	++	+	-
Coumarin	++	-	-
Terpenoids	+	-	+
Glycosides			

Table 2. shows that O.D. Value of Total phenolic, Total flavonoid, Total tannin content of *A. indicum*

Sample	Extract	Phenolic	Flavonoid	Tannin
A. indicum	Aqueous	2.2	1.8	1.2
	Acetone	8.9	5.6	2.5
	Ethanol	21.6	23.2	4.5

Table.3. Free radical scavenging activity of different extracts of *A. indicum*

S.No	Sample	IC_{50} µg/ml
1	Ascorbic acid	214
2	Ethanolic extract of *A.indicum*	375
3	Acetone extract of *A. indicum*	200
4	Aqueous extract of *A. indicum*	175

Fig.1: Shows FTIR results of ethanolic extracts of *A. indicum*