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Extraction, Phytochemical Screening and Quantitative Determination of Phenols and Flavonoids in Extract of *Kalanchoe pinnata* and *Pongamia pinnata*

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ABSTRACT

Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Secondary constituents contain alkaloids, flavonoids, phenol, saponin, steroids and tannins. Medicinal plants have anticancer, antimicrobial, antidiabetic, antidiuretic and anti-inflammation activities. The increasing interest in powerful biological activity of secondary metabolites outlined the necessity of determining their contents in medicinal plants. *Pongamia pinnata* (L.) Pierre (*P. pinnata,* Fabaceae) popularly known as Karanj or Karanja in Hindi, and Indian beech in English, is a medium-sized glabrous tree. Traditionally, different parts of *P. pinnata* such as bark, leaves, seeds, roots, flowers and stem have been utilized in the native medicine systems of different civilizations. *Kalonche pinnata (K. pinnata,* Crassulaceae*)* known as Patharchata in hindi, is a succulent plant. It has many pharmacological uses like a suitable treatment in sleep problems in cancer patients. Also called as wonder of life *K. pinnata* is used to treat diabetic foot infections. The aim of the present study is to examine leaf of *K. pinnata* and seed of *P. pinnata* for phytochemical profile. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by Folins Ciocalteau reagent method and aluminium chloride method respectively. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids, fixed oil and fats. The total phenolics content of hydroalcoholic extract *of K. pinnata* was (0.952 mg/100mg), followed by flavonoids (0.640mg/100mg) respectively. The total flavonoids content of hydroalcoholic extract of *P. pinnata* was (1.398mg/100mg). The present study concluded that the crude extract of *K. pinnata* and *P. pinnata* is a rich source of secondary phytoconstituents which impart significant antioxidant potential. The findings of the present study will be helpful to phytochemists, pharmacologists and pharmaceutical industries.

Keywords: *Pongamia pinnata, Kalonche pinnata,* Phytochemical, Folins ciocalteau reagent

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INTRODUCTION

Medicinal plants are wealthy source of novel drugs that forms the ingredients in traditional system of medicine, modern medicines, pharmaceutical intermediates and lead compounds in synthetic drugs[1]. The reason for using them as medicine lies in the fact that they contain chemical components of therapeutic value[2]. These compounds are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. The medicinal value of plants lies in some chemical substances (usually secondary metabolites) that produce a definite physiological action as the human body. In recent times focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems[3] including treatment against hepatocellular carcinoma[4]. Herbal medicines are being used by nearly about 80% of the world population, primarily in developing countries for primary health care[5]. Assessing the current status of health care system, inadequacies of synthetic drugs are likely to be more glaring in the coming years. In the present study, we have concentrated on the preliminary screening, quantitative determination, and the qualitative separation of secondary metabolites from leaves of selected ten different medicinal plants. Several plants have been studied for quantification of secondary metabolites, such as *Jatropha*[6], *Clerodendron colebrookianum* and *Zingiber cassumunar*[7], *Spondias mombin*[8] and leaves of *S. hyderobadensis*[9]. *Pongamia pinnata,* locally known as karanja, is a mangrove plant belonging to the family Fabaceae. It is a medium size glabrous tree with a

short bole and attaining a height of around 18 meter and its habitat is in the littoral regions of south-east Asia, Australia and Fiji[10,11]. Traditionally its bark is used in pile; leave are effective as medicated bath and rheumatic pains; and the seeds are used in hypertension, bronchitis, whooping cough, skin diseases and rheumatic arthritis[12-14]. Roots are used for cleaning gums, teeth, and ulcers also effective in gonorrhea[15,16]. Flowers used for diabetes. In ayurveda and unani medicine, used as anti inflammatory, antiplasmodial, anti-noneceptive, anti-hyperglycemic, anti-lipodoxidative, antidiarrheal, anti-ulcer, anti-hyper ammonic and antioxidant[17]. *Kalonche pinnata*, known as Patharchata in hindi, is a succulent plant belonging to the family Crassulaceae. *K. pinnata* has many pharmacological uses like a suitable treatment in sleep problems in cancer patients [18]. Also called as wonder of life *K. pinnata* is used to treat diabetic foot infections [19]. The plant develops a wound periderm in leaves when exposed to UV-B light and this tissue protects the plant from the stress condition [20]. The dichloromethane fraction of the steam distillate of *K. pinnata* leaves has shown excellent insulin secretagogue action and thus can be used in Diabetes mellitus[21]. *K. pinnata* is also found suitable in sleep related problems. The results encourage further clinical investigations on the sleep related problems[22]. The aqueous leaf extract of *K. pinnata* has demonstrated antihistaminic and expectorant activity in rodent models[23].The aim of this work was to determine the quality (types), quantity (amount) of bioactive compounds of leaf of *K. pinnata* and seed of *P. pinnata*.

MATERIAL AND METHOD

Plant material

The leaf of *K. pinnata* and seed of *P. pinnata* were collected from local area of Bhopal (M.P.) in the month of Jan, 2018.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine- Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Extraction

Dried powdered leaf of *K. pinnata* and seed of *P. pinnata* has been extracted with hydroalcoholic solvent using Soxhlet extraction process for 48 hrs, filtered and dried using vaccum evaporator at 40^0C. Finally the percentage yields were calculated of the dried extracts.

Qualitative phytochemical analysis of plant extract

The *K. pinnata* and *P. pinnata* extracts obtained was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate[24,25]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Test for carbohydrates

Molisch's test: In a test tube containing extract of drug, added two drop of freshly prepared 20% alcoholic solution of α- napthol and mixed concentrated sulphuric acid along the sides of the test tube. If carbohydrate present purple color or reddish violet color produce at the junction between two liquids.

Benedict's test: In a test tube containing extract of drug add benedict's solution, mix well, boiled the mixture vigorously for two minutes and then cooled. Formation of red precipitate due to presence of carbohydrates.

Barfoed's test: The barfoed's solution added to 0.5 ml of solution under examination, heated to boil. Formation of red precipitate of copper oxide was indicated the presence of carbohydrates.

Anthrone test: To the two ml of anthrone test solution, add the extract of drug. A green or blue colour indicated the presence of carbohydrate.

Test for alkaloids

Dragendorff's Test: Few mg of extract of the drug dissolved in 5 ml of water added 2 M hydrochloric acid until an acid reaction occurred; 1 ml of dragendorff's reagent (potassium bismuth iodide solution) was added an orange red precipitate indicated the presence of alkaloids.

Wagner's test: Acidify the extract of drug with 1.5 % v/v of hydrochloric acid and added a few drop of Wagner's reagent (iodine potassium iodide solution). Formations of reddish brown precipitate indicated the presence of alkaloids.

Mayer's Test: Two ml of extract solution was treated with 2 - 3 drops of Mayer's reagent was added (potassium mercuric iodide solution) formation of dull white precipitate indicated the presence of alkaloid.

Hager's Test: Extract of the drug solution was treated with 3 ml of Hager's reagent (saturated solution of picric acid) formation of yellow precipitate confirmed the presence of alkaloids.

Test for glycosides

Legal's test: Extract solution dissolved in pyridine then sodium nitroprusside solution was added to it and made alkaline. Pink red colour indicated the presence of glycosides.

Baljet's test: To the drug extract, sodium picrate solution was added, yellow to orange colour was indicated the presence of glycosides.

Borntrager's test: Few ml of dilute sulphuric acid solution, the test solution of extract was added. It was filtered and the filtrate was boiled with ether or chloroform. Then organic layer was separated to which ammonia was added, pink, red or violet colour was produced in orange layer confirmed the presence of glycosides.

Keller Kiliani test: Methanolic extract was dissolved in glacial acetic acid containing trace of ferric chloride one ml concentrated sulphuric acid was added carefully by the side of the test tube. A blue colour in the acetic acid layer and red colour at the junction of the two liquid indicated the presence of glycosides.

Test of saponins

1 ml of alcoholic extract was diluted with 20 ml distilled water and shaken in graduated cylinder for 15 minutes. One cm layer of foam indicated the presence of saponins.

Test for flavonoids

Shinoda test: In the test tube containing alcoholic extract of the drug added 5 - 10 drops of dil. hydrochloric acid followed by the small piece of magnesium. In presence of flavonoids a pink, reddish pink or brown color was produced.

Test for tannins

To the sample of the extract, ferric chloride solution was added appearance of dark blue or greenish black colour indicated the presence of tannins.

To the sample of extract, potassium cyanide was added, deep red colour was confirmed the presence of tannins.

To the sample of extract, potassium dichromate solution was added, yellow precipitate was produced.

Test for protein and amino acid

Biuret's test: To 2 - 3 ml of the extract of drug added in 1 ml of 40 % sodium hydroxide solutions and 2 drops of 1 % copper sulphate solution mix thoroughly, a purplish - violet or pinkish - violet colour produced that indicates the presence of proteins.

Ninhydrin's test: Two drops of freshly prepared 0.2 % ninhydrin reagent was added to the extract and heated to boiling for 1 - 2 min. and allow cooling. A blue colour developed that indicating the presence of proteins, peptides or amino acids.

Xanthoprotein test: To the extract in a test tube, add conc. nitric acid. A white precipitate was obtained and upon heating turns to yellow and cool the solution carefully. Added 20 % of sodium hydroxide solution in excess orange colour indicated presence of aromatic amino acid.

Millon's test: The small quantity of extract of the drug dissolved in distilled water added 5 - 6 drop of millon's reagent. A white precipitate was formed which turned red on heating, indicated the presence of proteins.

Lead acetate test: The extract was taken and two ml of 40 % sodium hydroxide solution was added and boiled, glacial acetic acid was added and cooled than added 1 ml of lead acetate solution, gray black precipitate was formed which indicated presence of sulphur containing amino acid.

Test of fats or fixed oils

Using sodium hydroxide: The extract was mixed in one ml 1 % of copper sulphate solution then added 10 % sodium hydroxide solution a clear blue solution was obtain which showed glycerin present in sample.

Using sodium hydrogen sulphate: The extract was taken in test tube added a pinch of sodium hydrogen sulphate pungent odour was formed which showed glycerin present in sample.

Saponification: Four ml of 2 % sodium carbonate solution was taken and the extract was added. Shaked vigorously and boiled. A clean soapy solution was formed cooled and added few drops of conc. HCl and observed that fatty separate out and float up.

Quantification of secondary metabolites

Quantitative analysis is an important tool for the determination of quantity of phytoconstituents present in plant extracts. For this TPC and TFC are determined. Extracts obtained from leaf of *K. pinnata* and seed of *P. pinnata* plant material of subjected to estimate the presence of TPC and TFC by standard procedure.

Total phenol determination

The total phenolic content was determined using the method of Olufunmiso *et al*[26]. A volume of 2ml of each extracts or standard was mixed with 1 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The colour developed was read at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

Total flavonoids determination

The total flavonoid content was determined using the method of Olufunmiso *et al*[26]. 1ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

RESULTS AND DISCUSSIONS

The crude extract so obtained after the Soxhlet extraction process, extract was further concentrated on water bath evaporation the solvent completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts was depicted in the table 1.

Table 1 Percentage yield of hydroalcoholic extracts of *kalanchoe pinnata* and *pongamia pinnata*

S. No.	*Kalanchoe pinnata* (Leaf)	*Pongamia pinnata* (Seed)
1.	4.8	3.7

The results of qualitative phytochemical analysis of the crude powder of leaf of *K. pinnata* and seed of *P. pinnata* are shown in Table 2 &3. Hydroalcoholic extracts of *K. pinnata* and *P. pinnata* showed the presence of flavonoids, phenols, saponins and diterpins.

Table 2 Phytochemical screening of *kalanchoe pinnata* (leaf) extract

S. No.	Constituents	Hydroalcoholic extract
.	**Alkaloids** Dragendroff's test Hager's test	 -ve -ve
.	**Flavonoids** Lead acetate Alkaline test	 +ve +ve
.	**Phenolics** $Fecl_3$	 +ve
.	**Proteins and Amino acids** Xanthoproteic test	 +ve
.	**Carbohydrates** Fehling's test	 -ve
.	**Saponins** Foam test	 +ve
.	**Diterpenes** Copper acetate test	 +ve

Table 3 Phytochemical screening of *pongamia pinnata* (seed) extract

S. No.	Constituents	Hydroalcoholic extract
.	**Alkaloids** Dragendroff's test Hager's test	 -ve -ve
.	**Flavonoids** Lead acetate Alkaline test	 -ve +ve
.	**Phenolics** $Fecl_3$	 -ve
.	**Proteins and Amino acids** Xanthoproteic test	 +ve
.	**Carbohydrates** Fehling's test	 +ve
.	**Saponins** Foam test	 +ve
.	**Diterpins** Copper acetate test	 -ve

The determination of the total phenolic content, expressed as mg gallic acid equivalents and per 100 mg dry weight of sample. The total flavonoids content of the extracts was expressed as percentage of quercetin equivalent per 100 mg dry weight of sample. TPC and TFC of hydroalcoholc extract of *K. pinnata* showed the content values of 0.952 and 0.640 respectively. The total flavonoid content of *P. pinnata* hydroalcoholc extract showed the content values of 1.398. Results are provided in (Table 4 and Fig. 1, 2).

Table 4 Total Phenolic and Total flavanoid content of hydroalcoholic extracts of *kalanchoe pinnata* and *pongamia pinnata*

S. No.	Hydroalcoholic extracts	Total Phenol (GAE) (mg/100mg)	Total flavanoid (QE) (mg/100mg)
1.	***Kalanchoe pinnata***	0.952	0.640
2.	***Pongamia pinnata***	-	1.398

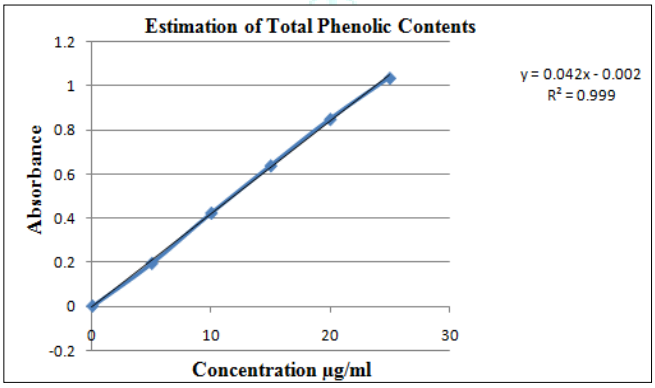

Figure 1 Graph of estimation of total phenolic content

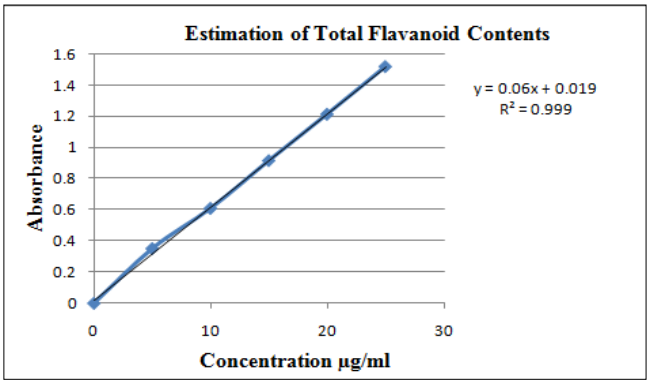

Figure 2 Graph of estimation of total flavonoid content

CONCLUSION

Qualitative and quantitative analysis of phenolics and flavonoids from *K. pinnata* and *P. pinnata* was achieved first time in this work. The observed level of phytoconstituents revealed that *K. pinnata* and *P. pinnata* is a rich source of antioxidant compounds. Currently available synthetic antioxidants are suspected to cause or prompt negative health effects, hence strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Moreover, the plant parts may be used as an alternative source for flavonoids and phenols for traditional remedies. Further phytochemical studies are also required to isolate and characterize active ingredients that are responsible for its antioxidant activity and to explore the existence of synergism if any, among the compounds.

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