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Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvents

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Abstract
In the present study, 10 medicinally important plant species were screened for their phytochemicals (quantitatively) by using 4 different solvent (water [AQ], Acetone [AE], Petroleum Ether [PE] and chloroform [CF]) extracted from their selected parts (leaves, stem, pericarp of the fruit and seeds cotyledons). All the plants which are selected for the study contains phytochemicals like alkaloids, flavonoids, steroids, phenols and saponins. The highest concentrations of alkaloids are observed in *L.officinale* leaf and *F.vulgare* stem extracts by using PE. The highest amounts of flavonoids are seen in AQ and PE extracts of *G.indica, D.loureiri, S. saponaria.* The moderate concentrations of phenols are reported in AQ and PE extracts of *J.curcas* and *S.saponaria* plant species. The high concentrations of steroids are reported in *S.saponaria* plant fruit pericarpic extract with PE. The concentration of phytochemicals varied, when different organic solvents are used for the extraction procedure.

Keywords: Phytochemicals, medicinal plants, bioactive compounds, flavonoids, saponins.

Introduction
Medical plants are plants containing built in active ingredients familiarized to cure disease and relieve from pain [1]. The use of traditional medicines and medicinal plants in mainly developing countries as remedial agents for the maintenance of health has been broadly observed [2]. Modern-day pharmacopoeia however contains at least 25% drugs derived from plants and many others, which are synthetic analogues, built on prototype chemical substances isolated form plants. Involvement in medicinal plants as a re-budding health assistance has been fuelled with the rising charges of prescription drugs in the safeguarding of personalized health and well being and the bio prospecting of new plant derived drugs [3]. The ongoing development recognition regarding medicinal plants is due to various reasons; include increasing faith in herbal medicine [4]. On the top of that, an increasing dependence on the use of these medicinal plants in the industrialized organizations has been traced towards the extraction and development of drugs and chemotherapeutics from these plants as well as from conventionally used herbal remedies [5]. The therapeutic properties of plants could be based on their anti-oxidant, anti-microbial, antipyretic effects of the phytochemicals constituents in them [6]. According to World Health Organization, medicinal plants would be the greatest source to obtain an array of drugs. Thus, such plants should be investigated to better understanding for their properties, safety practices in addition to usefulness [7].
In India, the ayurvedic system has features a numerous of such medicinal remedies on plants or plant products and the determination of their morphological, pharmacological or pharmacognostical characters can provide a better understanding of their active principle and mode of action. However a large number of tropical plants have not recently been studied in detail for their chemical constituents. So, in this regard we focused on phytochemical aspects in 10 selected indigenous plants of India: 1)*Garcinia indica* [GI] (leaves); 2) *Jatropha curcas* [JC] (leaves);3) *Nigella sativa* [NS] (leaves); 4) *Levisticum officinale* [LO] (leaves); 5) *Dracaena loureiri* [DL] (leaves) 6); *Woodfordia fruticosa* [WF] (stem);7) *Vaccinium macrocarpon*[VM](leaves) 8) *Foeniculum vulgare* [FV] (stem); 9) *Sapindus saponaria* [SS] (pericarp) ; 10)*Annona squamosa* [AS] (seeds).

Materials and Methods
Collection of Plant material
The plants were collected from their natural habitat, form different parts of south and north India. The plant material was identified and authenticated in the Department of Botany, P.B.Siddhartha College, Vijayawada, A.P. India.

Chemicals: The entire chemicals used in the present study are of analytical grade.

Preparation of plant extract

The collected plant material was carefully washed under running tap water followed by sterilized distilled water, and was air dried at room temperature in laboratory for 30-45 days. These dried plant materials were then homogenized to a fine coarse powder using an electric blender and then stored in air tight containers until further use. Various organic solvents viz. water [AQ], Acetone [AE], Petroleum ether [PE], and Chloroform [CF] were used for extractions. 10 gm of homogenized coarse powders of leaf, stem, pericarp and seed were soaked in different conical flasks containing 100 ml of water [AQ], Acetone [AE], Petroleum ether [PE], and Chloroform [CF] each and were allowed to stand for 30 min on a water bath with occasional shaking, which were then kept on rotary shaker at 200rpm for 24h [8-9]. Finally each sample extract (water [AQ], Acetone [AE], Petroleum ether [PE], and Chloroform [CF]) were prepared by using Soxhlet apparatus and was filtered through sterilized Whatman No 1 filter paper and concentrated to dryness under vacuum at 40^0C using rotaevaporater. Thus the obtained dried extracts were stored at 4^0C in labeled and stored in sterile bottles [10-11].

Quantitative Determination of Phytochemicals

Quantitative Estimation of Alkaloids

To 1ml of test extract 5 ml pH 4.7 phosphate Buffer was added and 5 ml BCG solution and shake a mixture with 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without extract. Atropine is used as a standard material and compared the assay with Atropine equivalents.

Quantitative Estimation of flavanoids

Total flavonoid content was determined by Aluminium chloride method using catechin as a standard. 1ml of test sample and 4 ml of water were added to a volumetric flask (10 ml volume). After 5 min 0.3 ml of 5 % Sodium nitrite, 0.3 ml of 10% Aluminium chloride was added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically. Results were expressed as catechin equivalents (mg catechin/g dried extract).

Quantitative Estimation of Saponins

Test extract were dissolved in 80% methanol, 2ml of Vanilin in ethanol was added, mixed well and the 2ml of 72% sulphuric acid solution was added, mixed well and heated on a water bath at 60^0c for 10min, absorbance was measured at 544nm against reagent blank. Diosgenin is used as a standard material and compared the assay with Diosgenin equivalents.

Quantitative Estimation of Steroids

1ml of test extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70 ± 2^0C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

Quantitative Estimation of Phenoilc Compounds

The total phenolics content in different solvent extracts was determined with the Folin- Ciocalteu's reagent (FCR)**.** In the procedure, different concentrations of the extracts were mixed with 0.4 ml FCR (diluted 1:10 v/v). After 5 min 4 ml of sodium carbonate solution was added. The final volume of the tubes were made up to 10 ml with distilled water and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer. A calibration curve was constructed using catechol solutions as standard and total phenolic content of the extract was expressed in terms of milligrams of catechol per gram of dry weight and the standard graph.

Results

Quantitative determination of phytochemical constituents

The ten selected plants species are subjected to quantitative analysis by standard methods. All the extracts which were prepared from the various organic solvents from selected parts of these ten plants are analysed for alkaloids, flavonoids, phenols, steroids and saponins.

Quantitative analysis of AQ Extract

The results obtained from the quantitative analysis of AQ extracts of all the selected 10 medicinal plants showed the presence of phytochemicals from highest to least extent. The table 1 result clearly indicated that the highest amount of alkaloids (180.98 µg/mg extract) are reported in plant *S.saponaria* and least amount of 68.25 µg/mg extract was observed in the seed cotyledonal AQ extract of *A.squamosa.* The plants *G.indica*, *N.sativa, W.fruticosa, and V.macrocarpon* AQ extract of selected parts showed the absences of alkaloids. The highest amounts of flavonoids are reported in *S.saponaria* fruit pericarpic of AQ extract with 198.48 µg/mg of dry weight. The least values of flavonoids are observed in *W.fruticosa* (87.43µg/mg). The flavonoids concentrations of all the selected plant parts of AQ extract were in the range of 87.43- 198.38 µg/mg dry weight of the extract. When the phenols concentrations are analysed only two plants *J.curcas* and *S.saponari* showed its presence with 68.24 µg/mg and 85.37µg/mg dry weight. The other plants reported the absence of phenols. When the AQ extract was quantitatively determined for the steroids, the plant source *F.vulgare* showed the highest amount of 68.39 µg/mg dry weight and *G.indica* reported 30.45 µg/mg. The concentrations of steroids are in the range of 30.45- 68.39 µg/mg. Finally the concentrations of saponins were determined for the AQ extract. The saponins are in the range of 30.03-157.32µg/mg. The plant pericarp of *S.saponaria* showed highest amounts of saponins (157.32 µg/mg) and least concentrations are observed in *L.officinale*. All the results obtained for these phytochemical (alkaloides flavonoids, phenols, steroids and saponins) are compared with standard chemicals Atropine, Catechin, Catechol, Cycloartenol and Diosgenin respectively.

Table 1: Quantitative determination of Alkaloid, Flavonoids, Phenols, Steroids and Saponins in Aqueous Extract of 10 selected medicinal Plants

S.No	Plant Source	Alkaloids	Flavonoids	Phenols	Steroids	Saponins
1	*Garcinia indica* [GI]	-	90.29	-	30.45	45.26
2	*Jatropha curcas*[JC]	110.29	120.45	68.24	-	32.52
3	*Nigella sativa* [NS]	-	112.32	-	67.24	43.27
4	*Levisticum officinales* [LO]	120.98	100.27	-	-	30.03
5	*Dracaena loureiri* [DL]	98.75	198.38	-	45.38	-
6	*Woodfordia fruticosa* [WF]	-	87.43	-	-	39.63
7	*Vaccinium macrocarpon*[VM]	-	135.25	-	-	33.39
8	*Foeniculum vulgare* [FV]	119.37	115.33	-	68.39	47.68
9	*Sapindus saponaria* [SS]	180.19	198.48	85.37	-	157.32
10	*Annona squamosa* [AS]	68.25	-	-	-	63.88

Quantitative analysis of AE Extract

The results obtained from the quantitative analysis of AE extracts of all the selected 10 medicinal plants showed the presence of phytochemicals from highest to least extent. The Table 2 result clearly indicated that the highest amount of alkaloids (197.62 µg/mg extract) are reported in plant *F.vulgare* and least amount of 100.52 µg/mg extract was observed in the seed cotyledonal of AE extract of *A.squamosa.* The plants *G.indica*, *N.sativa, Woodfordia fruticosa, and V.macrocarpon* of AE extract of selected parts showed the absences of alkaloids. The highest amount of flavonoids are reported in S.saponaria fruit pericarpic of AE extract with 165.53 µg/mg of dry weight. The least values of flavonoids are observed in *W.fruticosa* (45.89 µg/mg). The flavonoids concentrations of all the selected plant parts of AE extract were in the range of 45.89 - 165.53 µg/mg dry weight of the extract. When the phenols concentrations are analysed only two plants *J.curcas* and *S.saponari* showed its presence with 43.20 µg/mg and 98.78 µg/mg dry weights. The other plants reported the absence of phenols. When the AE extract was quantitatively determined for the steroids, the plant source *S.saponaria* showed the highest amount of 68.75 µg/mg dry weight and *L.officinale* reported 47.45µg/mg. The concentrations of steroids are in the range of 47.45-68.75µg/mg. Finally the concentrations of saponins were determined for the AE extract. The saponines are in the range of 23.48- 140.52 µg/mg. The plant pericarp of *S.saponaria* showed highest amounts of saponins (140.52 µg/mg) and least concentrations are observed in *L.officinale (23.48* µg/mg).

Table 2: Quantitative determination of Alkaloid, Flavonoids, Phenols, Steroids and Saponins in Acetone Extract of 10 selected medicinal Plants

S.No	Plant Source	Alkaloids	Flavonoids	Phenols	Steroids	Saponins
1	*Garcinia indica* [GI]	-	92.45	-	60.39	66.68
2	*Jatropha curcas*[JC]	120.38	110.29	43.20	-	38.27
3	*Nigella sativa* [NS]	-	87.45	-	49.70	23.46
4	*Levisticum officinales* [LO]	113.50	120.43	-	47.45	28.39
5	*Dracaena loureiri* [DL]	133.45	99.90	-	58.80	-
6	*Woodfordia fruticosa* [WF]	-	45.89	-	-	42.35
7	*Vaccinium macrocarpon*[VM]	-	135.75	-	-	98.47
8	*Foeniculum vulgare* [FV]	197.62	-	-	-	37.66
9	*Sapindus saponaria* [SS]	170.29	165.53	98.78	68.75	140.52
10	*Annona squamosa* [AS]	100.58	98.22	-	-	57.98

Quantitative analysis of PE Extract

The results obtained from the quantitative analysis of PE extracts of all the selected 10 medicinal plants showed the presence of phytochemicals from highest to least extent. The Table 3 result clearly indicated that the highest amount of alkaloids (198.73 µg/mg) are reported in plant *F.vulgare* and least amount of 98.25 µg/mg was observed in *V.macrocarpon* of PE extract. The *G.indica, N.sativa, D.loureiri, W.fruticosa* and *A.squamosa* of selected parts showed the absences of alkaloids. The highest amounts of flavonoids are reported in *G.indica* of PE extract with 198.25 µg/mg of dry weight. The least values of flavonoids are observed in *A.squamosa* (59.68µg/mg). The flavonoids concentrations of all the selected plant parts of PE extract were in the range of 59.68 – 198.25 µg/mg dry weight of the extract. The phenols compounds are totally absent in all the plants sources analysed in PE extract. When the PE extract was quantitatively determined for the steroids, the plant source *S.saponaria* showed the highest amount of 112.33 µg/mg dry weight and *V.macrocarpon* reported 32.98µg/mg. The concentrations of steroids are in the range of 32.98- 112.33µg/mg. Finally the concentrations of saponins were determined for the PE extract. The saponins are in the range of 12.57- 92.54 µg/mg. The plant pericarp of *S.saponaria* showed highest amounts of saponins (92.54µg/mg) and least concentrations are observed in *J.curcas* (12.57 µg/mg).

Table 3: Quantitative determination of Alkaloid, Flavonoids, Phenols, Steroids and Saponins in Petroleum Ether Extract of 10 selected medicinal Plants

S.No	Plant Source	Alkaloids	Flavonoids	Phenols	Steroids	Saponins
1	*Garcinia indica* [GI]	-	198.25	-	57.23	28.42
2	*Jatropha curcas*[JC]	100.50	158.76	-	-	12.57
3	*Nigella sativa* [NS]	-	186.60	-	80	54.55
4	*Levisticum officinales* [LO]	198.73	156.24	-	-	30.54
5	*Dracaena loureiri* [DL]	-	180.45	-	78.33	25.25
6	*Woodfordia fruticosa* [WF]	-	-	-	69.30	47.98
7	*Vaccinium macrocarpon*[VM]	98.32	-	-	32.98	25.50
8	*Foeniculum vulgare* [FV]	183.17	156.40	-	-	34.76
9	*Sapindus saponaria* [SS]	175.29	-	-	112.33	92.54
10	*Annona squamosa* [AS]	-	59.68	-	50.17	61.28

Quantitative analysis of CE Extract

The results obtained from the quantitative analysis of CE extracts of all the selected 10 medicinal plants showed the presence of phytochemicals from highest to least extent. The Table 4 result clearly indicated that the highest amount of alkaloids (180.52 µg/mg and 180.36µg/mg extract) are reported in plant *S.saponaria* and *F.vulgare* and least amount of 40.32 µg/mg was observed in the seed cotyledonal of CF extract of *A.squamosa.* The plants *G.indica*, *N.sativa, Woodfordia fruticosa, and V.macrocarpon* of CF extract of selected parts showed the absences of alkaloids. The highest amounts of flavonoids are reported in *S.saponaria* fruit pericarpic of CF extract with 160.12 µg/mg of dry weight. The least values of flavonoids are observed in *W.fruticosa* (40.25 µg/mg). The flavonoids concentrations of all the selected plant parts of CF extract were in the range of 40.25 – 160.12 µg/mg dry weight of the extract. When the phenols concentrations are analysed only one plants *S.saponari* showed its presence with 49.50 µg/mg dry weights and all other plants reported the absence of phenols. When the CF extract was quantitatively determined for the steroids, the plant source *G.indica* showed the highest amount of 68.52 µg/mg dry weight and *L.officinale* reported 25.42 µg/mg. The concentrations of steroids are in the range of 25.42- 68.52 µg/mg. Finally the concentrations of saponins were determined for the CF extract. The saponines are in the range of 20.21- 87.39 µg/mg. The plant pericarp of *S.saponaria* showed highest amounts of saponins (87.39 µg/mg) and least concentrations are observed in *L.officinale* (21.25µg/mg).

Table 4: Quantitative determination of Alkaloid, Flavonoids, Phenols, Steroids and Saponins in Chloroform Extract of 10 selected medicinal Plants

S.No	Plant Source	Alkaloids	Flavonoids	Phenols	Steroids	Saponins
1	*Garcinia indica* [GI]	-	148.23	-	68.52	-
2	*Jatropha curcas*[JC]	99.39	120.32	-	-	32.34
3	*Nigella sativa* [NS]	-	62.35	-	33.45	20.21
4	*Levisticum officinales* [LO]	110.58	78.27	-	25.42	21.29
5	*Dracaena loureiri* [DL]	95.35	80.23	-	30.75	26.28
6	*Woodfordia fruticosa* [WF]	-	40.25	-	-	20.19
7	*Vaccinium macrocarpon*[VM]	-	50.67	-	40.21	35.78
8	*Foeniculum vulgare* [FV]	180.36	-	-	-	49.59
9	*Sapindus saponaria* [SS]	182.52	160.12	49.40	69.20	87.34
10	*Annona squamosa* [AS]	40.32	110.16	-	-	33.36

Discussion

Plants play important roles in discovery associated with new beneficial therapeutic agents and have received significant focus because of their bio- active substances like antioxidants, hypoglycemic and hypolipidemic factors. India has a prosperous record associated with applying different potent natural herbs and plant based components regarding management of different diseases. Plants have invariably been exemplary source of drugs and a number of currently available drugs happen to be derived directly or indirectly from them. Flavonoids tend to be most commonly known with regards to antioxidant nature. They are transformers which alter the body biochemical reactions to carcinogenic chemicals, viruses, and things that trigger allergies. Many plants display their characters for anticancer, anti-inflammatory, antibacterial and anti-allergic nature [12], and could be useful in therapeutic roles [13]. Alkaloids tend to be organic and natural ingredients that have nitrogen, and are also physiologically active together with sedative and analgesic roles. They are found in reducing stress and depression symptoms. Alkaloids tend to be poisonous when taken in bulk amount due to their stimulatory effects, producing excitation associated with cell and nerve disorders [13-14]. Phenolic compounds are some of the most widespread molecules among plant secondary metabolites, are known to act as natural antioxidants [15]. Additionally, they serve as flower pigments, act as constitutive protection agents against invading organisms. Saponins are extensively utilized in veterinary vaccines because their character as an adjuvant and helps in the improvement of immune response. Many of them are useful in intracellular histo-chemistry staining permitting antibody access to intracellular protein molecules. The results extracted from our research are usually in agreement with the studies associated with other workers in the same field [16-19].

Conclusion

The plant based bio-active compounds have the effective dosage response with minimal side effects, when compared to the synthetic compounds. The studies conducted on these 10 selected plants species: 1)*Garcinia indica* [GI] (leaves); 2) *Jatropha curcas* [JC] (leaves);3) *Nigella sativa* [NS] (leaves); 4) *Levisticum officinale* [LO] (leaves); 5) *Dracaena loureiri* [DL] (leaves) 6); *Woodfordia fruticosa* [WF] (stem);7) *Vaccinium macrocarpon*[VM](leaves) 8) *Foeniculum vulgare* [FV] (stem); 9) *Sapindus saponaria* [SS] (pericarp) ; 10)*Annona squamosa* [AS] (seeds) showed the presences of phytochemicals. The presence of phytochemicals (secondary metabolites) is responsible for their therapeutic effects. It further reflects a hope for the development of many more novel therapeutic agents or templates from such plants which in future may serve for the production of synthetically improved therapeutic agents.

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