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## Quantitative phytochemical analysis of some medicinal plant seed by using various organic solvents

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#### Abstract

In the present work, six medicinally important plants seeds (*Acacia catechu*, *Sida cordifolia*, *Momordica foetida*, *Albizia procera*, *Mesua ferrea* and *Lantana camara*) were screened for their phytochemical quantitatively by using U-V Visible spectrophotometer by using 4 different solvent (water [AQ], Acetone [AE], Petroleum Ether [PE] and chloroform [CF]). The quantitative analysis of alkaloids, flavonoids, phenols, steroids, saponins and tannins showed the concentration of these seed extracts revealed the presences of different phytochemical compounds like alkaloids, flavonoids, phenols, steroids, saponins and tannins in appreciable amounts. The concentration of these phytochemicals varied from species to species and solvent to solvent in these extracts.

**Keywords:** phytochemical analysis, medicinal plant seed, organic solvents, Petroleum Ether.

#### Introduction

Various therapeutic properties are assigned to natural herbs. Medicinal plants comprise the principle source of new pharma drugs as well as health related products [1]. The history associated with plants used intended for therapeutic goal is probably since outdated as the background associated with humanity. Extractions as well as characterization associated with numerous active phytochemicals via these natural producers have given birth to higher profile drugs [2]. An expanding system associated with evidence indicates that secondary plants metabolites perform vital roles in human health and could possibly be nutritionally crucial [3]. Phytochemical screening process associated with medicinal plants revealed the existence for many substances which includes alkaloids, tannins, flavonoids, steroids, glycosides, saponins etc. Numerous plants extract components as well as phytochemicals present antioxidant / free radical scavenging properties [4-5]. Secondary metabolites associated with medicinal plants provide protection mechanism against predation through quite number of microbes, insects as well as herbivores [6]. Phytochemical studies regarding acetone, petroleum Ether and chloroform extracts in indigenous medicinal plants of *A. catechu*, *S. cordifolia*, *M. foetida*, *A. procera*, *M. ferrea* and *L. camara* are scanty. So, the study was taken up for the evaluation of plant secondary metabolites in these plants.

#### Materials and Methods: Collection of Plant material

The plants were collected from their natural habitat, from different parts of south and north India. The plant material was identified and authenticated.

#### Chemicals

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

#### Preparation of plant extract

The collected plant material (seeds) was carefully washed under running tap water followed by sterilized distilled water, and was air dried at room temperature in laboratory for 10-25 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. 100 gm of homogenized coarse powders of seeds 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 [7-9]. 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

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concentrated to dryness under vacuum at 40 °C using rotaevaporater. Thus the obtained dried extracts were lyophilized, labelled and stored at 4 °C 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, 2 ml of Vanilin in ethanol was added, mixed well and the 2 ml of 72% sulphuric acid solution was added, mixed well and heated on a water bath at 60 °C for 10 min, 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, 2 ml) 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 °C 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 upto 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.

**Determination of total tannins:** 500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M FeCl<sub>3</sub> in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min [12].

### Results

#### Quantitative determination of phytochemical constituents:

##### Quantitative analysis of AQ Extract

The results obtained from the quantitative analysis of AQ extracts of all the selected six medicinal plants showed the presence of phytochemicals from highest to least extent. The Table 1 result clearly indicated that the highest amount of alkaloids (113.52 µg/mg extract) are reported in plant *S.cordifolia* and least amount of 72.56 µg/mg extract was observed in the seed cotyledonal AQ extract of *L. camare*. The plants seed AQ extract of *A. procera* and *M. ferrea* showed the absences of alkaloids. The highest amounts of flavonoids are reported in *A. catechu* seed AQ extract with 120.29 µg/mg of dry weight. The least values of flavonoids are observed in *M. ferrea* (93.76 µg/mg). The flavonoids are absent in the AQ extract of *S. cordifolia*, *M. foetida* and *L. camare*. The plant seed extract of *M. ferrea* showed 93.76 µg/mg of flavonoid content. When the phenols concentrations are analysed only one plant *L. camare* showed its presence with 79.70 µg/mg. The other plants reported the absence of phenols. When the AQ extract was quantitatively determined for the steroids, the plant source *S. cordifolia* showed the highest amount of 92.26 µg/mg dry weight and next to it *M.ferrea* reported 80.40 µg/mg. The concentrations of steroids are in *M. foetida* is in the range of 62.76 µg/mg. The concentrations of saponins were determined for the AQ extract for all the six medicinal plant species. The saponins are in the range of 47.57 – 90.20 µg/mg. The plant seed extract of *A. catechu* showed highest amounts of saponins (90.20 µg/mg) and least concentrations are observed in *A. procera* (47.57 µg/mg). Finally when all the six seed AQ extracts were analysed for their concentration of tannins the plants *A. catechu* and *S.cordifolia* showed the absence of tannins. The highest concentrations of tannins are observed in *M. foetida* (82.45 µg/mg) and least was observed in *L. camare* (54.52 µg/mg). The plants *A. procera* and *M. ferrea* showed 66.20 and 79.80 µg/mg respectively. 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 six selected medicinal Plants

S.No	Plant Source	ALK	FL	PHE	ST	SP	TN
1	<i>Acacia catechu</i>	80.25	120.29	-	-	90.20	-
2	<i>Sida cordifoila</i>	113.52	-	-	92.26	-	-
3	<i>Momordica foetida</i>	96.73	-	-	62.76	69.34	82.45
4	<i>Albizia procera</i>	-	100.52	-	-	47.57	66.20
5	<i>Mesua ferrea</i>	-	93.76	-	-	51.90	79.80
6	<i>Lantana camare</i>	72.56	-	79.70	-	65.25	54.52

### Quantitative analysis of AE Extract

The results obtained from the quantitative analysis of AE extracts of all the selected six medicinal plants showed the presence of phytochemicals from highest to least extent. The Table 2 result clearly indicated that the highest amount of alkaloids (96.37  $\mu\text{g}/\text{mg}$  extract) are reported in plant *S.cordifolia* and least amount of 65.76  $\mu\text{g}/\text{mg}$  extract was observed in the seed cotyledonal AE extract of *L. camare*. The plant seed AE extract of *A. procera* showed the absences of alkaloids. The remaining plants alkaloids concentrations are in the range of 89.80- 70.25  $\mu\text{g}/\text{mg}$ . The highest amounts of flavonoids are reported in *M. ferrea* seed AE extract with 110.92  $\mu\text{g}/\text{mg}$  of dry weight. The least values of flavonoids are observed in *S. cordifolia* (54.95  $\mu\text{g}/\text{mg}$ ). The flavonoids are absent in the AE extract of *M. foetida* and *L. camare*. The plant seed extract of *A. procera* showed 96.25  $\mu\text{g}/\text{mg}$  of flavonoid content. When the phenols concentrations are analysed only three plant *A. procera*, *M.ferrea* and *L. camare*

showed its presence with 20.22, 40.55 and 52.33  $\mu\text{g}/\text{mg}$ . The other plants reported the absence of phenols. When the AE extract was quantitatively determined for the steroids, the plant source *L.camare* showed the highest amount of 60.25  $\mu\text{g}/\text{mg}$  dry weight and next to it *M.foetida* reported 50.70  $\mu\text{g}/\text{mg}$ . The concentrations of steroids in *A.procera* is in the range of 39.40  $\mu\text{g}/\text{mg}$  and the remaining three plants (*A.catechu*, *S.cordifolia* and *M.ferrea*) showed that absences of steroids. The concentrations of saponins were determined for the AE extract for all the six medicinal plant species. The saponins are in the range of 34.55 – 66.76  $\mu\text{g}/\text{mg}$ . The plant seed extract of *S.cordifolia* showed the absences of saponins. Finally when all the six seed AE extracts where analysed for their concentration of tannins the plants *A. catechu* and *A.procera* showed the absence of tannins. The highest concentrations of tannins are observed in *M. ferrea* (89.75  $\mu\text{g}/\text{mg}$ ) and least was observed in *L. camare* (40.39  $\mu\text{g}/\text{mg}$ ).

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

S.No	Plant Source	ALK	FL	PHE	ST	SP	TN
1	<i>Acacia catechu</i>	74.62	93.59	-	-	34.55	-
2	<i>Sida cordifoila</i>	96.37	54.95	-	-	-	77.93
3	<i>Momordica foetida</i>	89.80	-	-	50.70	56.70	65.42
4	<i>Albizia procera</i>	-	96.25	20.22	39.40	60.75	-
5	<i>Mesua ferrea</i>	70.25	110.92	40.53	-	52	89.75
6	<i>Lantana camare</i>	65.76	-	52.31	60.25	66.76	40.39

### Quantitative analysis of PE Extract

The results obtained from the quantitative analysis of PE extracts of all the selected six medicinal plants showed the presence of phytochemicals from highest to least extent. The Table 3 result clearly indicated that the highest amount of alkaloids (85.35  $\mu\text{g}/\text{mg}$  extract) are reported in plant *S.cordifolia* and least amount of 59.60  $\mu\text{g}/\text{mg}$  extract was observed in the seed cotyledonal PE extract of *L. camare*. The plants seed PE extract of *A. procera* showed the absences of alkaloids. The highest amounts of flavonoids are reported in *A. catechu* seed PE extract with 97.52  $\mu\text{g}/\text{mg}$  of dry weight. The least values of flavonoids are observed in *M. foetida* (30.25  $\mu\text{g}/\text{mg}$ ). The flavonoids are absent in the PE extract of *M. ferrea* and *L. camare*. When the phenols concentrations are analysed *S.cordifolia* and *M. foetida* reported the absence of

phenols. The concentrations of phenols for the other four plans are in the range of 36.44-60.75  $\mu\text{g}/\text{mg}$ . When the PE extract was quantitatively determined for the steroids, the plant source *A. procera* showed the highest amount of 70.90  $\mu\text{g}/\text{mg}$  dry weight and next to it *A. catechu* reported 60.30  $\mu\text{g}/\text{mg}$ . The concentrations of saponins were determined for the PE extract for all the six medicinal plant species. The saponins are in the range of 30.29 – 54.25  $\mu\text{g}/\text{mg}$ . The plant seed extract of *L.camare* showed highest amounts of saponins (54.25  $\mu\text{g}/\text{mg}$ ) and least concentrations are observed in *M.ferrea* (30.29  $\mu\text{g}/\text{mg}$ ). Finally when all the six seed PE extracts where analysed for their concentration of tannins the plant *A. procera* showed the absence of tannins. *A. catechu* showed highest concentration of 99.72  $\mu\text{g}/\text{mg}$  and the least was observed in *L. camare* (69.52  $\mu\text{g}/\text{mg}$ ).

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

S.No	Plant Source	ALK	FL	PHE	ST	SP	TN
1	<i>Acacia catechu</i>	69.70	97.52	60.75	60.30	40.25	99.72
2	<i>Sida cordifoila</i>	85.35	49.63	-	58.76	-	80
3	<i>Momordica foetida</i>	78.53	30.25	-	40.80	-	72
4	<i>Albizia procera</i>	-	95.33	36.44	70.10	-	-
5	<i>Mesua ferrea</i>	80	-	40.39	-	30.29	84.99
6	<i>Lantana camare</i>	59.60	-	40.90	-	54.25	69.52

### Quantitative analysis of CF Extract

The results obtained from the quantitative analysis of CF extracts of all the selected six medicinal plants showed the presence of phytochemicals from highest to least extent. The Table 4 result clearly indicated that the highest amount of alkaloids (80.59  $\mu\text{g}/\text{mg}$  extract) are reported in plant *S.cordifolia* and least amount of 60.52  $\mu\text{g}/\text{mg}$  extract was observed in the seed CF extract of *L. camare*. The plant seed CF extract of *A. procera* showed the absences of alkaloids.

The highest amounts of flavonoids are reported in *M.ferrea* seed CF extract with 92.57  $\mu\text{g}/\text{mg}$  of dry weight. The least values of flavonoids are observed in *S.cordifolia* (47.53  $\mu\text{g}/\text{mg}$ ). The flavonoids are absent in the CF extract of *M. foetida* and *L. camare*. When the phenols concentrations are analysed only four plants showed its presence with range of 30.63- 57.53  $\mu\text{g}/\text{mg}$ . The other plants (*S.cordifolia* and *M.foetida*) reported the absence of phenols. When the CF extract was quantitatively determined for the steroids, the plant

source showed the highest amount of 30.42-52.90 µg/mg dry weight. The concentrations of saponins were determined for the CF extract for all the six medicinal plant species. The saponins are in the range of 20.39 – 60.48 µg/mg. The plant seed extract of *A.procera* showed the absences of saponins.

Finally when all the six seed CF extracts were analysed for their concentration of tannins the plants *S.cordifolia* and *A.procera* showed the absence of tannins. The highest concentrations of tannins are observed in *A.catechu* (76.33 µg/mg) and least was observed in *L. camare* (37.89 µg/mg).

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

S.No	Plant Source	ALK	FL	PHE	ST	SP	TN
1	<i>Acacia catechu</i>	70	80.30	57.53	30.42	60.48	76.33
2	<i>Sida cordifolia</i>	80.59	47.53	-	-	20.39	-
3	<i>Momordica foetida</i>	76.50	-	-	40.76	40.32	45.54
4	<i>Albizia procera</i>	-	86.33	40.50	52.90	-	-
5	<i>Mesua ferrea</i>	73.39	92.57	33.39	-	40.78	75.20
6	<i>Lantana camare</i>	60.52	-	30.63	50.73	50.60	37.89

## Discussion

The result extracted from our research on all the six medicinal plants seed with four solvents (AQ, AE, PE and CF) are usually in agreement with the studies of [13-27]. Arrays of phytochemicals were detected in all the test extracts extracted from seeds by different solvents. These phytochemicals included: alkaloids, flavonoids, cardiac glycosides, phenols, terpenoids, tannins, steroids, and saponins. They are normally produced by plants as an evolutionary adaptation to harsh environment or in response to attack by other organisms [28]. They however have been found to inadvertently confer antimicrobial protections to humans due to compounds synthesized in the secondary metabolism [29] as well as being immuno-modulative [30-31].

## 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 6 selected plants species: (*Acacia catechu*, *Sida cordifolia*, *Momordica fotida*, *Albiziz procera*, *Mesua ferrea* and *Lantana camare*.) 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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