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Effect of Solvents on Extraction of Certain Medicinal plant Polyphenols

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ABSTRACT

The present study revealed that, acetone among pure forms of experimental solvents and Ethyl acetate + Methanol + Water among the mixture forms could extract significantly higher ($p<0.01$) amount of polyphenols from all the plant leaves. The amount of polyphenols extracted in mixture forms of solvents were significantly higher ($p<0.01$) than those in their pure forms for majority of the plant leaves. It is concluded that, solvents in their mixture forms were better to extract phyto-phenols from leaves of the medicinal plants.

Keywords: solvents, leaves, medicinal plants, polyphenols

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INTRODUCTION

Oxidation is an ongoing process in aerobic life which produces free radicals by electron transfer to initiate Haber Weiss chain reactions where cell damage becomes the root cause of pathogenesis of various diseases. Antioxidants such as thiols, ascorbic acid and polyphenols either slow down or prevent the process by removing free radicals as reducing agents through auto-oxidation. Plants are the richest source of polyphenols which act as natural safe anti-oxidants to prevent mainly diseases originated out of oxidative stress[1] such as cardio-vascular diseases and cancer by modulating the cause beyond its origin. Therefore, phenolic compounds in medicinal herbs have gained importance due to their high anti-oxidative activity[2] and are exploited much for drug development in human and veterinary medicine.

The biosynthesis and concentration of polyphenols in plants depends on climatic conditions, harvest seasons, post-harvest treatments, physiological state during collection, agricultural and environmental factors[3]. The polyphenol contents in different plants may vary between cultivars as well as within the individual plant. Besides, plant polyphenols are secondary metabolites / derivatives / isomers of flavones, isoflavones, flavonols, catechins and phenolic acids[4,5] and includes over 8,000 structural variants[6]. The solubility of different components of phyto-phenols also differs between solvents resulting variable concentration of polyphenols in the extracts of different solvents obtained from a single plant or its part. So, the present project was designed to study the effect of solvents in pure and their mixture forms on the concentration of total polyphenols in the leaves of different medicinal plants.

MATERIALS AND METHODS:

Experimental plants:

The following locally available folk medicinal plants were selected for the study after identification and classification following the description of Saxena and Brahman[7]. The leaves of these plants were collected from apparently healthy plants at pre-flowering stage from different regions of Orissa province.

Local name	Botanical name	Local name	Botanical name
Barun	*Crateva nurvala*	Kantabheji	*Solanum violaceum*
Basanga	*Adhatoda vasica*	Kanteikoli	*Flacouritia indica*
Begunia	*Vitex negundo*	Kusum	*Scheleichera oleosa*
Bhalia	*Semecarpus anacardium*	Kumbhi	*Careya arborea*
Jayasandha	*Litsea glutinosa*	Chara	*Buchananja litifolia*
Mahula	*Madhuca indica*	Pedipedika	*Abutilon indicum*

Leaf powder:

Fresh leaves were cleaned, dried under shade, ground into fine powder and kept in a glass jar under cool and dry condition for preparation of extracts.

Solvent:

The study was classified into seven groups basing on the solvents used for extraction of polyphenols from the leaves of the experimental plants as per the following details.

List of solvent used of extraction

Groups	Solvents used for extraction
A	Water
B	Methanol
C	Ethanol
D	Acetone
E	Methanol (70%) + Water (25%) + Acetic acid (5%)
F	Ethyl acetate (60%) + Methanol (30%) + Water (10%),
G	Acetone (90%) + Water (9.5%) + Acetic acid (0.5%).

Extraction:

Extraction of dried leaf was done in closed system by Anton Par-Multi wave 3000-801-V of Microwave Assisted Extraction System by taking 2gm of ground leaf and 20ml of solvent in each vessel at 80^0 C for 25 minutes followed by 15 minutes cooling. The extract was filtered through a filter paper before removal of chlorophyll by hexane treatment.

Removal of chlorophyll:

Equal volume of filtered extract and hexane were vertexed in a wide mouth test tube for 2 minutes and allowed to stand undisturbed for some time. Superficial fluid was pipetted out as hexane treated sample without chlorophyll.

Estimation of total polyphenol:

The concentration of total polyphenol in the extract was determined and results were expressed as Gallic acid equivalents[8]. Samples (0.2 ml) were mixed with 1.0 ml of 10-fold diluted Folin–Ciocalteu reagent and 0.8 ml of 7.5% sodium carbonate solution. After standing for 30 min at room temperature the absorbance was measured at 765 nm UV-visible spectrophotometer against 0.2 ml of methanol: water (6:4) as the blank.

Statistical analysis:

The data was subjected to analysis of variance to test the significance of difference of mean values between different groups according to Snedecor and Cochran[9] **.**

RESULTS AND DISCUSION:

The polyphenolic contents in the study varied between the solvents from pure forms to their mixtures within a plant and between the plants also. The phenolic contents in the leaves of

Crateva nurvala, Adhatoda vasica, Solanum violaceum, Scheleichera oleosa, Abutilon indicum and *Buchananja litifolia* in pure solvent extracts of water, methanol and ethanol did not vary significantly between Gr-A, B and C respectively. The leaves of *Vitex negundo, Semecarpus anacardium, Madhuca indica* and *Flacouritia indica* exhibited significantly higher ($p<0.01$) concentrations of total phenolics in methanol extracts at Gr-B than those of aqueous extracts in Gr-A. Ethanolic leaf extracts of *Vitex negundo, Semecarpus anacardium* and *Madhuca indica* exhibited significantly higher polyphenols at Gr-C than those in aquous extracts at Gr-A. Similarly, acetone in Gr-D extracted significantly higher ($p<0.01$) amount of polyphenol in the leaves of *Madhuca indica* and *Flacouritia indica* than the values at Gr-A, B and C. Among the pure form solvents at Gr-A, B, C and D, acetone extracted higher concentrations of phenolics in the leaves of all plants where as water was observed to extract lower amounts in comparison to other solvents under study. The amounts of polyphenols extracted in the solvents at Gr-A, B, C and D were in the order of acetone > methanol > ethanol > water.

Variation in the polyphenolic content is related to the biosynthesis in the plants which is influenced by cultivar, climate, postharvest treatments and agricultural and environmental factors. The variation of the polyphenolic contents between the plants may be due to phytochemical diversity as reported earlier[10]

The organic solvents in their mixture form at Gr-E, F and G did not exhibit significant variation in extraction of polyphenols in the leaves of *Crateva nurvala, Vitex negundo* and *Abutilon indicum.* Significantly higher ($p<0.01$) amount of polyphenols were extracted by the solvent mixtures at Gr-F and G for the leaves of *Adhatoda vasica, Semecarpus anacardium, Madhuca indica* and *Flacouritia indica.* In addition, the rest of the plant leaves exhibited variable amounts of polyphenols in different solvent mixtures. Among three solvent mixtures, Ethyl acetate + Methanol + Water extract at Gr-F showed significantly higher ($p<0.05$) amount of polyphenols in all the leaves under study followed by the rest two mixtures in a variable manner for different plants.

Polyphenols being the secondary metabolites of plants (phytochemicals) most commonly include saponins, tannins, catechins, isocatechins, alkaloids, coumarin lignans, anthraquinones, anthocyanin, cardiac glycosides, cyanogenic glycosides and flavonoids of flavones and isoflavones[11,12] where 10,000 flavonoids, 12,000 alkaloids and 30,000 terpenoids contribute the composition of total phenolics of the plant[13,14,15]. The solubility of each polyphenolic component depends on physical property and chemical nature and varies from solvent to solvent. Majority of the components are soluble in organic solvents than in aqueous ones and this might be the reason

Table 1.Concentrations of total polyphenols as mg of Gallic acid Eqv / g of leaf in different plants extracted under experimental solvents (Mean ± SE)

Plant name	Gr-A	Gr-B	Gr-C	Gr-D	Gr-E	Gr-F	Gr-G
Crateva nurvala	1.26 ^{a} ±0.05	1.39^{a}±0.05	1.31 ^{a}±0.05	1.48 ^{b} ±0.05	1.83^{c}±0.05	1.86 ^{c} ±0.06	1.63 ^{c} ±0.06
Adhatoda vasica	1.67 ^{a} ±0.05	2.05^{a}±0.05	1.73 ^{a}±0.05	2.66 ^{b} ±0.07	2.62 ^{b} ±0.07	3.44 ^{c} ±0.08	2.98 ^{c} ±0.07
Vitex negundo	6.47 ^{a} ±0.14	8.40^{b}±0.16	7.31^{ab}±0.09	8.77 ^{b} ±0.15	9.33 ^{c} ±0.16	9.75 ^{c}±0.18	9.32 ^{c} ±0.14
Semecarpus anacardium	3.12 ^{a} ±0.07	4.95 ^{b} ±0.07	4.12 ^{ab}±0.07	5.45 ^{b} ±0.07	5.13 ^{b} ±0.07	6.12 ^{c} ±0.06	5.74 ^{c} ±0.08
Madhuca indica	5.98 ^{a} ±0.10	7.05 ^{b} ±0.12	6.84 ^{ab}±0.06	7.80 ^{c} ±0.14	7.24 ^{b} ±0.11	8.02 ^{c} ±0.14	7.74 ^{c} ±0.12
Solanum violaceum	2.89 ^{a} ±0.05	3.24 ^{a} ±0.06	2.54 ^{a}±0.06	3.57 ^{b} ±0.07	3.26 ^{a} ±0.07	3.78 ^{b} ±0.05	3.66 ^{b} ±0.08
Flacouritia indica	4.92 ^{a} ±0.07	5.56 ^{b} ±0.08	4.92 ^{a}±0.09	6.71 ^{c} ±0.08	6.04 ^{b} ±0.07	6.97 ^{c} ±0.06	6.66 ^{c} ±0.08
Scheleichera oleosa	8.81 ^{a} ±0.15	8.85 ^{a} ±0.11	8.41 ^{a}±0.10	9.89 ^{b} ±0.15	8.98 ^{a} ±0.14	10.31 ^{c} ±0.14	9.92 ^{b} ±0.15
Buchananja litifolia	1.11 ^{a}±0.03	1.42 ^{a} ±0.04	1.33 ^{a}±0.04	2.29 ^{b} ±0.05	2.63 ^{c} ±0.06	2.66 ^{c} ±0.06	2.16 ^{b} ±0.05
Abutilonindicum	1.32 ^{a}±0.03	1.35 ^{a} ±0.04	1.14 ^{a}±0.03	1.43 ^{a} ±0.05	1.76 ^{b} ±0.04	1.89 ^{b} ±0.05	1.58 ^{b} ±0.06

Means with different superscripts within rows showed significant difference ($p<0.01$) between the groups.

for extracting significantly higher amount of polyphenols in Gr-B, C and D solvents than in water at Gr-A. Similarly, the extraction in Gr-E, F and G depicts that more number of polyphenolic components are soluble in the mixtures of organic solvents than in aqueous and individual ones and it might be due to synergistic effect of component solubility of polyphenols.

CONCLUSION

(i) the amounts of polyphenols extracted in the solvents were in the order of acetone > methanol > ethanol > water and (ii) Ethyl acetate + Methanol + Water extract showed significantly higher amount of polyphenols in all the leaves under study followed by Acetone (90%) + Water (9.5%) + Acetic acid (0.5%) and Methanol (70%) + Water (25%) + Acetic acid (5%).

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