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Effect of Extraction Methods and Solvent on Phytochemical Composition of Medicinal Plant Extracts

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ABSTRACT

In the present study Tridax procumbens, Murraya koenigii and Pongamia pinnata leaves extracts of water, ethanol, methanol and acetone were prepared using 'Green extraction' methods such as microwave assisted and ultrasonic assisted solvent extraction. The extracts were screened for phytochemicals like alkaloids, phenolic compounds, flavonoids, tannins, terpenoids and diterpenoids. Further, phytochemical composition of microwave assisted extracts was compared with the ultrasonic assisted extracts. The results reveal that phytochemical composition of leaves extracts highly varied with the extraction method as well as solvent used for extraction. The present study may be useful to select an appropriate solvent and method for isolation of phytochemicals from T. procumbens, M. koenigii and P. pinnata leaves.

Keywords: *Tridax procumbens, Murraya koenigii, Pongamia pinnata*, Phytochemicals, Green extraction

INTRODUCTION

Tridax procumbens, Murraya koenigii and *Pongamia pinnata* are familiar medicinal plants in India (Figure 1) [1,2]. *T. procumbens* is a common medicinal herb belongs to family Astraceaae. The herb is used as indigenous medicine for variety of ailments such as bronchial catarrh, malaria, dysentery, diarrhea, stomach ache, headache [3]. Traditionally, *T. procumbens* leaves have been used for wound healing, as anticoagulant, antifungal and insect repellent. *P. pinnata* is a medium size tree belongs to family Leguminosae and widely distributed throughout tropical Asia. *P. pinnata* leaves were used to cure disorders like anthelmintic, digestive, inflammations, wounds and piles. *T. procumbens* leaves juice can be used for treatment of cold, cough, and diarrhea [4]. *M. koenigii* is a popular herb in India. The herb belongs to family Rutaceae. In Indian traditional system this herb is used to treat ailments such as vomiting, stomachache, dysentery, carminative, analgesic, antihelminthes and also used to cure piles, itching and inflammation [5,6].

The bioactivity of plant extracts is attributed to phytochemical constituents present in the extracts. However, phytochemical constituents of a plant extract is extremely dependent on the polarity of solvent, solvent to plant material ratio, particle size of plant material, temperature and extraction method. Several studies reported that the nature of solvents and extraction methods are the two important factors effecting the phytochemical composition of plant extracts [7,8]. Recent years, extraction methods namely Ultrasonic Assister Extraction (UAE) and Microwave Assisted Extraction (MAE) are considered as green extraction methods and extensively studied for extraction of phytochemical from different plant materials [9-11]. The results of previous studies indicated that MAE and UAE are efficient methods for extraction of phytochemicals from plant materials [12,13].

In the present work, the efficient extraction method for extraction of phytochemicals from *T. procumbens, M. koenigii* and *P. pinnata* leaves were investigated to assess the effect of solvent nature on phytochemical composition of *T. procumbens, M. koenigii* and *P. pinnata* leaves extracts, different solvents like water, ethanol, methanol and acetone extracts were prepared using MAE and UAE methods and then screened for commonly expected phytochemicals in leaves such as alkaloids, phenolic compounds, flavonoids, tannins, terpenoids and diterpenoids.

MATERIALS AND METHODS

Collection of plant materials

Fresh leaves of *Tridax procumbens, Murraya koenigii* and *Pongamia pinnata* were collected from in and around Andhra Loyola College campus, Vijayawada, Andhra Pradesh, India. The collected leaves were washed thoroughly under running tap water to remove dust particles and then washed with double distilled water. The leaves were dried in shade and grinded to fine powder using domestic mixture grinder. The leaves powders were stored in air tight container until it is used.

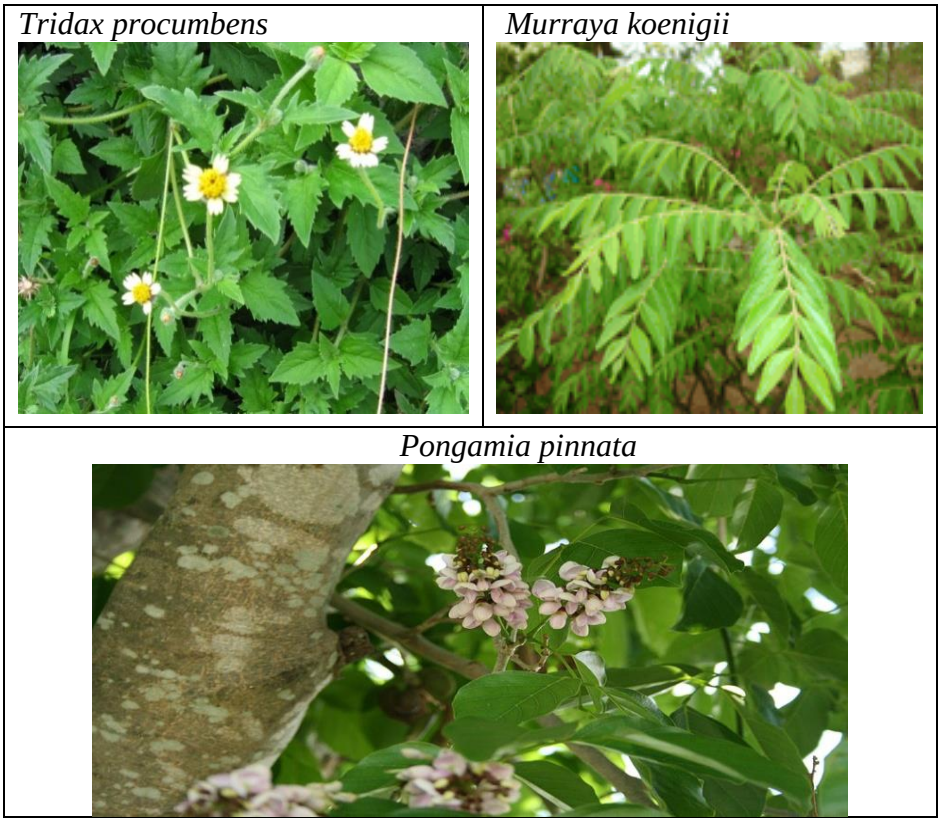

Figure 1: *Tridax procumbens, Murraya koenigii* and *Pongamia pinnata*

Preparation of extracts

The polar solvents like water, methanol, ethanol and acetone were selected as extraction solvents. Aqueous, methanol, ethanol and acetone leaf extracts of *T. procumbens, M. koenigii* and *P. pinnata* were prepared using ultrasonic assisted extraction (UAE) and microwave assisted extraction (MAE) methods.

Ultrasonic assisted extraction (UAE)

Exactly 1 g of leaves powder was immersed in 50 ml of water, methanol, ethanol and acetone separately and then placed in ultrasonic bath (Labman, Digital ultrasonic cleaner) for 2 h. The extracts were filtered and used further.

Microwave assisted extraction (MAE)

Exactly 1 g of leaves powder was immersed in 50 ml of water, methanol, ethanol and acetone separately and then heated in microwave oven (LG LCRT1513SB Countertop Microwave Oven) for 10 min with 1 min time intervals. The extracts were filtered and used further.

Preliminary phytochemical screening

Aqueous, methanol, ethanol and acetone extracts of *T. procumbens, M. koenigii* and *P. pinnata* leaves were analysed for various phytochemical constituents like alkaloids, phenolic compounds, flavonoids, tannins, terpenoids and diterpenoids. Standard methods reported in the literature were applied for phytochemical analysis [14,15].

Test for alkaloids

Wagner's test (Test-1)

To one ml of extract few drops of Wagner's reagent was added. Development of yellow color or brown precipitate indicates a positive test for alkaloids.

Mayer's test (Test-2)

One ml of Mayer's reagent was added to one ml of extract. Formation of yellowish buff colored precipitate indicates the presence of alkaloids.

Test for flavonoids

Shinoda's test

To 0.5 ml of extract few drops of concentrated HCl and small piece of magnesium wire were added and the solution was boiled for few minutes. Appearance of reddish pink color shows a positive test for flavonoids.

Test for phenolics

Ferric chloride reagent test

To 1 ml of the extract few drops of 5% aqueous ferric chloride solution was added. Appearance of intense color precipitate confirms a positive result for the presence of phenolics.

Test for terpenoids

Exactly 2 ml of extract was added to 2 ml of chloroform followed by careful addition of 3 ml of concentrated sulphuric acid. Formation of reddish brown color layer at interface of two layers indicated a positive result.

Test for diterpenoids

To 1 ml of extract few drops of 1% aqueous copper acetate solution was added. Formation of emerald green precipitate confirms a positive result for the presence of diterpenoids.

RESULTS AND DISCUSSION

Effects of extraction method and solvent on phytochemical composition

Phytochemical screening of water, methanol, and ethanol and acetone extracts of *T. procumbens*, *M. koenigii* and *P. pinnata* leaves prepared using MAE and UAE methods was performed and the results are presented in Tables 1-3 respectively. Phytochemical screening of different solvent extracts of *T. procumbens* leaves indicating the presence of alkaloids, phenolic compounds, flavonoids, tannins, terpenoids and diterpenoids. Similar to our results, Dhanabalan et al. reported the presence of alkaloids, tannin, flavonoids, terpenoids, phlobatannin, saponins, steroids and cardiac glycosides in methanol extract of *T. procumbens* leaves [16]. Rajaram et al. were also reported the presence of tannin, flavonoids, alkaloids, phenolics, diterpenes, anthocyanin, coumarins, saponin and steroid in acetone water extracts of *T. procumbens* leaves [17]. However, they were not investigated the efficient method for extraction of phytochemicals from *T. procumbens* leaves. In the present study, phytochemical composition of UAE and MAE extracts of *T. procumbens* were compared and the results were given in Table 1. As shown in Table 1, higher levels of flavonoids, terpenoids and diterpenoids were obtained in UAE extracts. The present investigation results clearly showed that UAE was an efficient method than MAE method for extraction of phytochemicals from *T. procumbens* leaves.

Table 1: Phytochemical screening of different extracts of *Tridax procumbens* leaves

Phytochemicals							
Extraction method MAE	**Alkaloids (Test-1)**	**Alkaloids (Test-2)**	**Phenolics**	**Flavonoids**	**Tannins**	**Terpenoids**	**Diterpenoids**
Water	-	+	-	-	++	-	+
Ethanol	+	++	+	+	+++	-	+
Methanol	++	+	+	++	+++	+	++
Acetone	++	++	++	++	++	-	+
Extraction method UAE	**Alkaloids (Test-1)**	**Alkaloids (Test-2)**	**Phenolics**	**Flavonoids**	**Tannins**	**Terpenoids**	**Diterpenoids**
Water	++	++	+++	++++	++++	++++	++++
Ethanol	+++	+++	++	++++	++++	++++	++++
Methanol	++++	++++	+++	++++	++++	++++	++++
Acetone	++	++	++	++++	+++	++++	++++

Phytochemical analysis of leaves extracts of *M. koenigii* proved the presence of flavonoids, alkaloids, phenolic compounds, tannins, terpenoids and diterpenoids. Studies on phytochemical composition of *M. koenigii* stem, leaves, bark and root have showed the presence of alkaloids and phenolic compounds at higher levels [18]. Baskaran et al. studied the effect solvent on phytochemical composition of extracts of *M. koenigii* leaves [19]. They prepared different solvent extracts of *M. koenigii* leaves using Soxhlet extraction method and screened for alkaloids, phenolic compounds, flavonoids, terpenoids, saponins, phytosterol and glycosides in water, methanol, ethanol, acetone ethyl acetate, chloroform, hexane and petroleum ether extracts. The authors reported that phytochemical composition of leaf extracts of *M. koenigii* dependent on the nature of solvent. It is well known that the phytochemical constituents of plant extracts vary with the polarity of solvent as well as the extraction method. Therefore, in the present work the influence of extraction methods on phytochemical composition of extracts of *M. koenigii* leaves was investigated by varying the solvents. The results presented in Table 2 shows that MAE was the best method as compared to UAE method for extraction of phytochemicals from *M. koenigii* leaves.

Table 2: Phytochemical screening of different extracts of *Murraya koenigii* leaves

Phytochemicals							
Extraction method MAE	**Alkaloids (Test-1)**	**Alkaloids (Test-2)**	**Phenolics**	**Flavonoids**	**Tannins**	**Terpenoids**	**Diterpenoids**
Water	-	+	+	+++	++++	+	+
Ethanol	++	++	++++	++++	++	++	+++
Methanol	++	+++	++++	++++	+++	++++	+++
Acetone	++	++	+++	++++	++	++	+++
Extraction method UAE	**Alkaloids (Test-1)**	**Alkaloids (Test-2)**	**Phenolics**	**Flavonoids**	**Tannins**	**Terpenoids**	**Diterpenoids**
Water	+	-	++	+++	++	+	++
Ethanol	+	-	+	++	-	++	++
Methanol	++	++	-	+	+++	++	+
Acetone	++	+++	-	+++	++	++	+

Qualitative analysis of various extracts of *P. pinnata* leaves indicating the presence of terpenoids, diterpenoids, tannins, phenolic compounds, flavonoids and alkaloids. Similarly Prashanth and Krishnaiah were also identified the phytocomponents like phenolic compounds, alkaloids, flavanoids, tannins, carbohydrates, triterpenoids and steroids in aqueous and ethanolic extracts of *P. Pinnata* leaves [20]. Sharmila and Shameema banu were undertaken the phytochemical analysis of acetone and chloroform extracts of *P. glabra* leaves [21].

Their results indicated that flavonoids, tannins and cardiac glycosides were present in acetone extract while alkaloids and saponins were present in the chloroform extracts of *P. glabra* leaves. In addition amino acids, proteins, terpenoids, reducing sugars, anthroquinones and steroid were absent in both the extracts. The reported results clearly prove that solvent selected for extraction significantly influences the phytochemical constituents and phytochemical constituents vary from plant to plant. Similar to previous results, the present results given in Table 3 infer that both solvent and extraction method influences the phytochemicals constituents of leaves extracts of *P. pinnata*. The highest phytochemical constituents were observed in MAE extracts.

Table 3: Phytochemical screening of different extracts of *Pongamia pinnata* leaves

Phytochemicals							
Extraction method MAE	**Alkaloids (Test-1)**	**Alkaloids (Test-2)**	**Phenolics**	**Flavonoids**	**Tannins**	**Terpenoids**	**Diterpenoids**
Water	-	-	+++	++++	++++	++++	++++
Ethanol	+	+	+	++++	++++	++++	++++
Methanol	+	+	++	++++	+++	++++	++++
Acetone	+++	+++	++	+++	++++	++++	++++
Extraction method UAE	**Alkaloids (Test-1)**	**Alkaloids (Test-2)**	**Phenolics**	**Flavonoids**	**Tannins**	**Terpenoids**	**Diterpenoids**
Water	-	-	-	++++	++++	++++	++++
Ethanol	+	+	+	++++	+++	++++	++++
Methanol	++	++	++	++++	+	+	++
Acetone	++	++	+	++++	-	-	++

From Tables 1 and 2, it can be seen that the phytochemical composition of *T. procumbens* and *M. koenigii* was higher in methanol (26.06%) than that of ethanol (17.03%) and acetone (12.33%). This shows that the efficiency of phytochemicals extraction from *T. procumbens* and *M. koenigii* leaves increases with increasing polarity of the solvent. It is intrusting to note that phytochemicals composition of water extracts of *T. procumbens* and *M. koenigii* leaves was found to be lower than the pure methanol extracts even though water polarity (25.58%) is higher than methanol. Similar results were reported for the extraction of phenolic compounds and flavonoids from *Limnophila aromatica* [22]. In case of *P. pinnata*, phytochemicals composition was highly dependent on the extraction method over the solvent used for extraction. The highest phytochemicals composition was observed in MAE extracts than in UAE extracts.

CONCLUSION

Phytochemical analysis of MAE and UAE extracts of *T. procumbens*, *M. koenigii* and *P. pinnata* leaves infer that the phytochemical composition vary significantly with extraction method and solvent. UAE method was found to be best method for extraction of phytochemicals from *T. procumbens* leaves whereas MAE was best for *M. koenigii* and *P. pinnata*. Pure methanol was found to be an efficient solvent for the extraction of phytochemicals from *T. procumbens*, *M. koenigii* and *P. pinnata* leaves as compared to ethanol, acetone and water. The present study may be useful to select an appropriate solvent and method for isolation of alkaloids, phenolic compounds, flavonoids, tannins, terpenoids and diterpenoids from *T. procumbens*, *M. koenigii* and *P. pinnata* leaves. Further studies are needed to isolate the phytochemicals from these plant leaves.

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