

Microwave assisted extraction of phenolic compounds and investigation on antioxidant activity

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Abstract:

Microwave assisted extraction has gained lot of attention due to its advantages such as less solvent consumption, short time period, higher extraction efficiency, therefore serves as better alternative for conventional extraction methods of plant materials. Plant phenolic compounds are important constituents responsible for reducing the oxidative stress that induces tissue damage which is the one of the major causative factors associated with the chronic disease. *Nyctanthes arbor-tristis* and *Tamarindus indica* are most commonly known as Parijata and Tamarind respectively and they hold an enormous part in ayurvedha for their wonderful and widespread health benefits in a broad area of applications. Hence the in the present study microwave assisted extraction of phenolic content from *Nyctanthes arbor-tristis* and *Tamarindus indica* was done and their antioxidant potential was investigated

In order to compare the extraction efficiency of phenolic compounds, Conventional extraction method i.e, Soxhlet extraction and Microwave assisted extraction methods were used to prepare the extracts of Parijata and Tamarind using ethanol as solvent. The prepared extracts were subjected to preliminary phytochemical analysis and FTIR. The total phenolic content was determined by using Folin-Ciocalteu method and in-vitro antioxidant activity was investigated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

Extracts of Parijat and Tamarind leaves showed the presence of steroids, alkaloids, saponins, carbohydrates, phenolic compounds by preliminary phytochemical analysis. FTIR spectrum of the extract showed characteristic peak at 3314.62 cm⁻¹, 1635 cm⁻¹ which provides evidence for presence of phenolic compounds. Microwave assisted extracts showed higher phenolic content and high antioxidant activity.

Key words: Parijat, Tamarind, Anti-oxidant, Total phenolic content, phytochemical analysis, UV spectroscopy, FTIR, DPPH assay

Introduction

In recent years, global trend is increasing towards the use of natural antioxidant in the area of food science and complementary medicines in comparison with synthetic antioxidants which are toxic to human health [1] Plants acts as rich source of natural antioxidants due to the presence of secondary metabolites mainly poly-phenolic compounds and flavonoids. Phenolic compounds acts as a reducing agents due to their redox potential, thereby acting as antioxidants, thereby have important role in lipid peroxidation [2].

Several extraction techniques such as Microwave extraction (MAE), supercritical fluid extraction, solvent extraction, Soxhlet extraction are used for extraction of antioxidant constituents such as Polyphenolic compounds from Plant [3-6]. Among these MAE is popularly being used due to its higher efficiency of extraction [7]. Many reports have shown that MAE has more extraction potential than conventional method of extraction [8,9].

Nyctanthes arbor-tristis also called as Night jasmine or Parijata is a small tree belongs to the family of Oleaceae The leaf juice of parijata is used as anthelmintic, antipyretic, to treat loss of appetite, piles, liver disorders, malaria, obstinate sciatica, rheumatism, and as a diaphoretic. Additionally, crushed leaves are used for ulcers and to reduce inflammation [10].

Tamarindus indica popularly known as imli or Tamarind belonging to the family of Leguminosae is widely used for their fruits . Bark of this plant is endowed with effects such as astringency, anti-diarrheal, anthelmintic, emmenagogue, tonic, thermogenic, anti-inflammatory, anti-fungal, diuretic. Fruits present with remedies for digestion, as laxatives, carminative, in ophthalmology, as an anti-septic and febrifuge [11].

Hence considering the medicinal importance of Tamarind and *Nyctanthes*, in the current study an effort was made to extract the phenolic compound by MAE including investigation of antioxidant potential of the plant extracts.

Materials and methods:

Collection and preliminary processing of plant material

Fresh leaves of Parijata and Tamarind were collected from locality of Mangalore. The leaves were washed with water to remove adhering dirt, shade dried and then ground into coarse powder.

Extraction of plant material

Extracts of the Parijata and Tamarind leaves were prepared separately by conventional method (Soxhlation) and by microwave assisted extraction (MAE) method.

Soxhlet Extraction was carried out by subjecting 10 grams of powdered leaf material to soxhlet extraction for a period of 36 hours with 350ml of ethanol as solvent at 50°C.

Microwave Assisted Extraction (MAE) was done by using 10 grams of the leaves was in a microwave oven (CATA-R) working at a 800W irradiation power and 2450MHz frequency. MAE was done using ethanol as solvent at a temperature of 50°C for a period of 5mins [12].

After the extraction, solutions were filtered, filtrate was evaporated and concentrated using rotary flash evaporator to get dry extracts. The extracts obtained by soxhlation and MAE compared for the percentage yield and amount of phenolic content, thereby indicating their antioxidant activity.

Preliminary Phytochemical Screening (Qualitative Analysis)

The ethanolic leaf extracts of Parijata and Tamarind were subjected to various phytochemical tests to determine the presence of various phyto-constituents [13].

Estimation of phenolic content

Phenolic content in Tamarind and Nyctanthes extracts were estimated by Folin-Ciocalteu method [14]. Test solution was prepared by taking 100mg of ethanolic extract was dissolved in 100ml of phosphate buffer (pH 6.8). 10ml of the above solution and diluted up-to 100ml with phosphate buffer. From this 4ml was transferred to 25ml volumetric flask to which 1.25ml of FC reagent and 2.5ml of sodium carbonate was added. The final mixture volume was adjusted with distilled water. For the calibration curve, Gallic acid was used as standard and standard solution of 50µg/ml was prepared in phosphate buffer (pH 6.8). Aliquots of 2, 4, 6, 8, 10 and 12 ml were taken in 6 different 25ml volumetric flasks from the stock solution. Into each, 1.25 ml of Folin-Ciocalteu reagent and 2.5ml of 20% sodium carbonate. The resulting blue colour was evaluated for absorbance at 765 nm on UV-Visible spectrophotometer (Shimadzu 1700) after keeping in dark at room temperature for 30min. Using the linear equation obtained from the standard plot, the phenolic content was estimated and depicted as gallic acid equivalent per gram of the plant material.

Fourier transfer Infrared spectroscopy

Fourier Transform Infrared spectroscopy can be considered as a powerful tool for identifying functional groups present in compounds. Ethanolic extract was encapsulated in KBr pellet of FT-IR to prepare translucent sample discs. The spectrum of these samples was recorded using Bruker FTIR spectrophotometer.

Determination of antioxidant activity by DPPH assay

The radical scavenging activity was determined by the use of DPPH free radical assay with some modification according to the method of Barros *et al.*[15]. Ascorbic acid was used as a standard by dissolving 10mg in 10ml of methanol as diluent. Serial dilutions were prepared using 10 µl, 20 µl, 30 µl, 40 µl and 50 µl of this standard and the volume made up-to 50 µl using methanol. To these 100 µl of DPPH was added and the absorbance was noted at a wavelength of 517nm after 30 minutes of incubation against blank taken as 50 µl of the diluent (methanol). The test solutions were also prepared in a similar manner using 10mg of the 4 plant extracts and dissolving in 10ml of methanol. 100 µl of DPPH reagent and 50 µl of methanol were used as the control. Using the following equation, the percentage inhibition activity was calculated:

$$\% \text{ Inhibition} = [(A_o - A_I) / A_o] \times 100 \dots \dots \dots (i)$$

Where A_o stands for the absorbance of the control, and A_I denotes the absorbance of the extract/ standard. All the readings were performed in triplicates.

Results and Discussion

Extraction of plant material and Phytochemical Analysis

The yield of the extract of Tamarind was found to be 26.67% and 64.45% from soxhlation and MAE respectively. Soxhlation of Parijata extract produces yield of 16.8% while MAE produced yield of 61.6 %. Hence MAE produced higher yield indicating that it has higher extraction efficiency when compare to the conventional Soxhlet extraction.

Preliminary phytochemical analysis of tamarind and parijata leaf extract confirmed the presence of alkaloids, glycosides, phenols, tannins, steroids, flavonoids.

5.3. Estimation Total phenolic content by Folin-Ciocalteu method

Amount of polyphenolic content in Tamarind and Nyctanthes extracts was estimated by applying the Folin-Ciocalteu method where gallic acid is opted as the standard. Absorbance of different concentration of gallic acid solutions was measured at 765 nm for preparation of standard plot and results of which is shown in **Fig.1**.

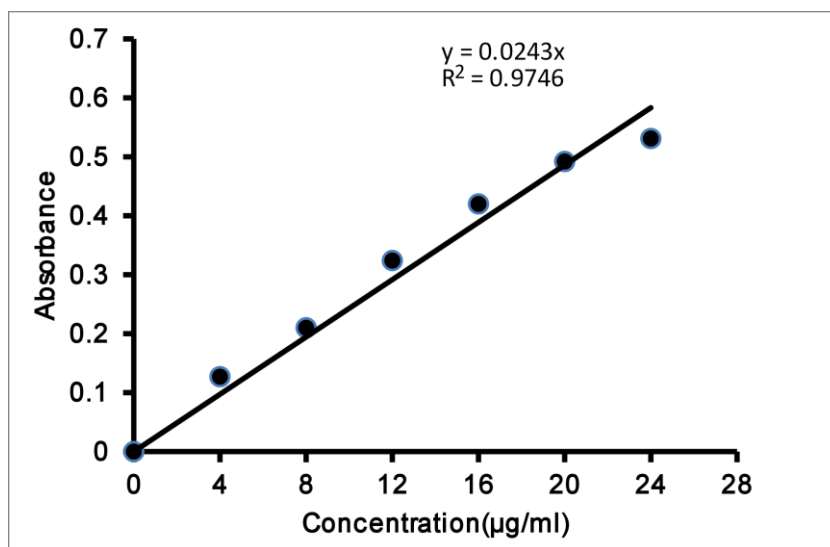


Fig. 1: Standard plot of gallic acid in phosphate buffer pH 6.8 at 765nm

From the calibration equation, amount of poly-phenolic content was found to be 180.0 and 149.17 mg/g Gallic acid equivalent (GAE) in tamarind extract obtained by MAE and soxhlation respectively. Extract of Parijata obtained from soxhlation and MAE was found to contain 529.83 and 640.43 mg/g Gallic acid equivalent (GAE) of phenolic content respectively.

Results showed that amount of phenolic content were higher in extracts obtained from MAE than soxhlation in both the plant parijata and tamarind. Hence MAE was found to be superior for getting high extraction efficiency of phenolic content than Soxhlet extraction. The phenolic content is higher in Parijata plant than in Tamarind.

Fourier transfer infrared spectroscopy: The FTIR analysis of extracts obtained from MAE was done to determine the important functional groups present. FTIR spectrum of Parijata and Tamarind leaf extracts are shown in **Fig 2&3**

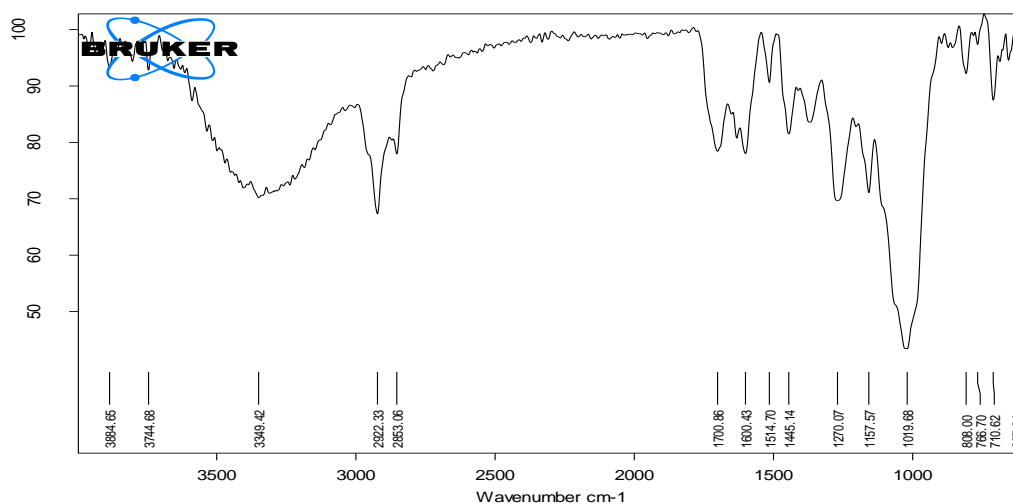


Fig.2: FTIR spectrum of Parijata leaf extract obtained by MAE

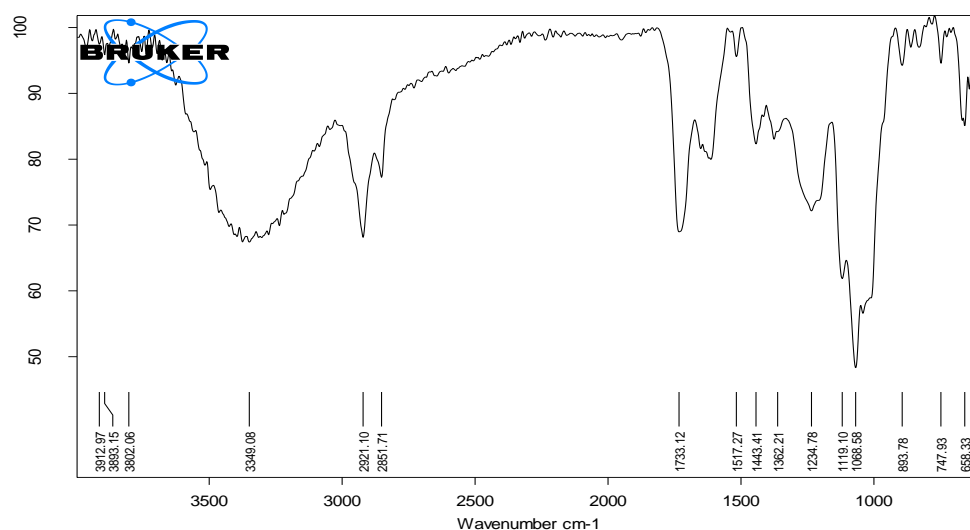


Fig.3: FTIR spectrum of Tamarind leaf extract obtained by MAE

The FTIR spectrum of the extracts showed characteristic band between 3338 cm^{-1} to 3342 cm^{-1} corresponding to the stretching vibration of $-\text{OH}$ and $-\text{H}$ bonded alcoholic and phenolic groups. Band at 1638 cm^{-1} is mainly due to aromatic $\text{C}=\text{C}$ and $\text{C}=\text{O}$ vibrations [16]. Hence FTIR confirms the presence of phenolic compounds in both tamarind and parijata extracts.

5.5. DPPH radical scavenging activity:

Free radical scavenging activity using the DPPH method of different concentration of Tamarind and parijata extracts are shown in **Table 1**. Result showed that as the concentration of the extracts was increased, DPPH radical scavenging activity also increased. At highest concentration ($1\mu\text{g}/\mu\text{l}$), tamarind and parijata extracts obtained from MAE showed higher percentage of DPPH scavenging activity i.e. 65.34% and 75.08% respectively, in comparison to extracts from soxhlation method.

Parijata plant extract showed significant antioxidant activity when compared to tamarind extract which proved that the antioxidant behaviour of plants mainly depends on the amount of phenolic content and it enhances the total antioxidant capacity of medicinal plants [17,18]

Table 1: Antioxidant activity of Tamarind and parijata extracts by DPPH assay

Samples	Concentration µg/µl	% DPPH Scavenging activity
Tamarind Extracts from Soxhlation	0.2	10.56671
	0.4	18.00472
	0.6	24.2621
	0.8	37.36718
	1.0	44.451
Tamarind Extracts from MAE	0.2	32.11334
	0.4	32.29044
	0.6	38.60685
	0.8	49.46871
	1.0	65.34829
Parijata Extracts from Soxhlation	0.2	9.208973
	0.4	23.78985
	0.6	37.24911
	0.8	49.11452
	1.0	68.53601
Parijata Extracts from MAE	0.2	20.30697
	0.4	40.90909
	0.6	67.47344
	0.8	73.19953
	1.0	75.08855
Standard	0.2	71.72373
	0.4	72.25502
	0.6	72.37308
	0.8	72.90437
	1.0	75.55726

Conclusions

Parijata leaves contain appreciable quantity of phenolic content, and act as potent free radical scavenger, hence it can be used as a source of natural antioxidants which will have higher potential in the treatment of various diseases arising due to involvement of free radical and hence could lead to a new field of future research. In comparison to soxhlation method, MAE showed higher extraction efficiency for phenolic compound and hence showing higher antioxidant potency. It has been proved that polarity of the solvent, nature of the extracted compounds and extraction process highly affects therapeutic activities of the plant extracts [19]. The antioxidant behaviour of the plant mainly depends on the amount of phenolic content and it enhances the total antioxidant capacity of medicinal plants [20,21]

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References

1. Sofidiya MO, Odukoya OA, Familoni OB, Inya-Agha SI. Free radical scavenging activity of some Nigerian medicinal plants. *Planta Medica*. 2006 ;72(11):171.
2. Correia Da Silva TB, Souza VK, Da Silva AP, Lyra Lemos RP, Conserva LM. Determination of the phenolic content and antioxidant potential of crude extracts and isolated compounds from leaves of *Cordia multispicata* and *Tournefortia bicolor*. *Pharmaceutical biology*. 2010 ;48(1):63-9.
3. Sandeep DS, Nayak P, Nayana K, Nasiha, Alfish N, Kumar N, Kumar A. Developing anti-dandruff shampoo formulations using different Indian plant Herbs- An Eco-friendly Hair care Cosmetic. *Journal of Xi'an Shiyou University, Natural Science Ed.*2021; 17(9):684-693.
4. Reshma R, Achala B , Rajesh KS, Harish , Raman R. Evaluation of Anti-venom Activity of Leaf Extract of *Wedelia trilobata*. *Journal of Xi'an Shiyou University, Natural Science Ed.*2021; 17(9):609-613.
5. Hashif A, Khandige PS, Nayak P. Evaluation of antidepressant activity of *Garcinia cambogia* on experimentally induced depression in Mice. *Journal of Xi'an Shiyou University, Natural Science Ed.*2021; 17(9):55-60
6. Olivia J, Varghese, Shetty P, Sharanya M. Skeletal muscle relaxant potential of *Annona reticulata* L. leaf extract in swiss albino mice a preclinical study. *Journal of Xi'an Shiyou University, Natural Science Ed.*2021; 17(9):673-683
7. Ganzler K., Salgo A., Valko K. Microwave extraction-a novel sample preparation method for chromatography. *J. Chromatogr.* 1986;371:299–306.
8. Pan X., Niu G., Liu H. Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. *Chem. Eng. Process.* 2003;42:129–133.
9. Hong N., Yaylayan V.A., Raghavan G.S.V., Paré J.R.J., Bélanger J.M.R. Microwave assisted extraction of phenolic compounds from grape seed. *Nat. Prod. Res.* 2001;15:197–204.
10. Agrawal J, Anirban Pal. *Nyctanthes arbor-tristis* Linn—A critical ethnopharmacological review . *J Ethnopharmacol* 2013;146: 645–658
11. Chimsah FA, Nyarko G, Abubakari AH. A review of explored uses and study of nutritional potential of tamarind (*Tamarindus indica* L.) in Northern Ghana. *African Journal of Food Science*. 2020 ;14(9):285-94.
12. Jyothi D, Khanum S, Sultana R. Optimisation of microwave assisted extraction of withanolides from roots of *Ashwagandha* and its comparison with conventional extraction method. *International Journal of Pharmacy and Pharmaceutical Sciences* 2010;2(4): 46-50.
13. Kokate CK, Purohit AP, Gokhale SB. *Pharmacognosy*. 10th ed. New Delhi: Nirali Prakashan Pvt Ltd; 1998,92-4.
14. Bukhari SB, Bhangar MI, Memon S. Antioxidative activity of extracts from fenugreek seeds (*Trigonella foenum-graecum*). *Pak J Anal Environ Chem*. 2008;9(2):78-83.
15. Oliveira RN, Mancini MC, Oliveira FCS, Passos TM, Quilty B, Thire RMS, McGuinness GB. FTIR analysis and quantification of phenols and flavonoids of five commercially available plants extracts used in wound healing. *Revista Materia*.2016; 21(3):767–79.
16. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. Antioxidant activity of plant extracts containing phenolic compounds. *J Agric Food Chem* 1999;47: 3954-62.
17. Hettige S. Salutary effects of *Carica papaya* leaf extract in dengue fever patients – a pilot study. *Srilankan Fam Physician*. 2008;29:17-19.

18. Bukhari SB, Bhanger MI, Memon S. Antioxidative activity of extracts from fenugreek seeds (*Trigonella foenum-graecum*). Pak J Anal Environ Chem. 2008;9(2):78-83.
19. Metrouh-Amir H, Duarte CMM, Maiza F. Solvent effect on total phenolic contents, antioxidant, and antibacterial activities of *Matricaria pubescens*. Ind Crop Prod. 2015;67:249–56.
20. Eberhardt MV, Lee CY, Liu RH. Antioxidant activity of fresh apples. Nature. 2000; 405:903-4.
21. Wang H, Cao G, Prior RL. Total antioxidant capacity of fruits. J Agricultural Food Chem. 1996;44(3):701-5.