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Research Article

QUANTIFICATION OF TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENTS IN EXTRACTS OF *OROXYLUM INDICUM L.*KURZ

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ABSTRACT

Crude extracts of different parts such as bark, leaves, petioles, seeds and fruitwall of *Oroxylum indicum* L.Kurz were used to determine the total phenolic and flavonoid contents. The amount of total phenols were analysed by using Folin-Ciocalteau assay and the amount of total flavonoids were analysed using aluminium chloride calorimetric assay. The results revealed that the total phenolic content (TPC) and total flavonoid contents (TFC) varied among the different parts isolated in various solvent extracts. Methanolic extract of seeds showed highest amount of TPC (155.0 mg GAE/100g) and less amount of TPC (26.0 mg GAE/100 g) was found in petroleum ether extract of bark where as TFC was found to be highest in methanolic extract of bark (207.5 mg CE/100 g) and less amount of TFC was recorded in petroleum ether extract of leaf (15.0 mg CE/100 g). The results presented here not only shows the TPC and TFC but also their ratio and distribution in different parts of the tree that could be used in the treatment of various ailments.

Keywords: Leaves, petioles, bark, seeds, fruitwall, phenols, flavonoids.

INTRODUCTION

Plants are the good sources for the discovery of pharmaceutical compounds and medicines. Natural products could be potential drugs for humans or live stock species and also these products and their analogues can act as intermediates for synthesis of useful drugs (Makkar et al, 2009). Plants possess many phytochemicals with various bioactivities including antioxidant,anti-inflammatory and anticancer (Wu, 2002) and one among such plants is *Oroxylum indicum* L.Kurz. *O.indicum* is an endangered medicinal forest tree widely used in the treatment of many ailments in ayurvedic, herbal and folk medicine. Different parts sucgh as seeds, leaves and bark of styem and root known to contain substantial amounts of phytoconstituents such as phenolics, flavonoids, tannins having the ability to inhibit the free radicals that are excessively produced, hence can act as antioxidants (Bauer, 2002). Several plants and vegetables used in traditional medicine can provide diverse secondary metabolites with antioxidant potentials most of which are isolated phenolic compounds (Ramarathnam et al 1997). Phenolics compounds are very important plant constituents exhibiting antioxiodant activity by inactivating lipid free radicals, or by preventing the decomposition of hydroperoxides into free radicals (Maisuthisakul etal 2007; Nasapon et al,2010). The continued search among plant secondary metabolites for natural antioxidants has gained importance in recent years because of the increasing awareness of herbal remedies as potential sources of phenolic oxidants(Aliyu et al 2010). It is well known that phenolic compounds contribute to quality and nutritional value in terms of modifying colour, taste, aroma and flavor besides providing health beneficial effects (Memnune sengul et al, 2009). The species of *Oroxylum indicum* (Bignoniaceae) is an endangered tree with medicinal and economic importance. It is recognized as it is multipurpose medicinal tree by ayurveda as it is used in many formulations such as *Shyonakapatpak, Bruhatpanchanulayadikwath, Dashmula* and *Chyawanprash* (Vaidya, 1975). All parts of this tree possesses medicinal values(Zavhoor Ahmad et al 2012) such as antirheumatic, (Rastogi and Mehrothra 1998; Pal and Jain 1998), antimicrobial, anti oxidant, antifungal, anti inflammatory, anti cancerous (Ali et al 1998). Hence the present work has been designed to investigate the total phenolic content (TPC) and total flavonoid contents (TFC) present in various solvent extracts of different parts of *O.indicum* due to its enormous therapeutic uses.

MATERIALS AND METHODS

Plant material

The leaves, petioles, fruits and stem bark were collected from Mallur Reserve Forest, Warangal District, Andhra Pradesh, India. The collected plant matertials were washed with running tap water to remove surface contaminants and finally air dried, then ground into fine powder using an electric blender and were stored in air tight containers until use.

Exrtraction of samples

Powdered plant materials (100g each) i.e, leaves, petioles, seeds, stem bark and fruit wall were soaked in different solvents such as methanol, chloroform, benzene, petroleum ether individually for a week. The extracts were then filtered through Whatman No.1. filter paper and the solvent was evaporated using rotatory evaporator. These crude extracts were stored in sealed containers at room temperature until use.

Chemicals and Reagents

Folin-Ciocalteau phenol reagent, gallic acid, anhydrous sodium carbonate, methanol, Deionised water, chloroform, benzene, petroleum ether, Sodiumnitrite, aluminium tri chloride, sodium hydroxide.

Preparation of sample

The crude extracts of each sample of 100 mg was weighed and phenolic and flavonoid compounds were extracted with 5.0 ml of different solvents such as chloroform, methanol, petroleum ether and benzene individually.

Determination of total phenolic content

The total phenolic content (TPC) of the crude extracts of leaves, petioles, seeds, bark of stem and fruit wall were determined using the method of Singleton *et al.*(1999) with slight modifications. To 0.5 ml of test sample,1.5 ml (1:10 v/v diluted with distilled water) Folin-Ciocalteau reagent was added and allowed to stand for 5 min at 22ºC. After 5 min, 2.0ml of 7.5% of sodium carbonate was added. These mixtures were incubated for 90 min in the dark with intermittent shaking. After incubation development of blue colour was observed. Finally absorbance of blue colour in different samples were measured at 725nm using colorimeter. The phenolic cotent was calculated as gallic acid equivalents GAE/g on the basis of standard curve of gallic acid. The results were expressed as Gallic acid equivalents (GAE)/g of the plant material. All the determinations were carried out six times.

Determination of total flavonoid content

The total flavonoid content(TFC) of different parts such as leaves, petioles, seeds, bark of stem and fruitwall of *O.indicum* was determined using the aluminium chloride assay through colorimetry. An aliquot (0.5 ml) of extracts were taken in different

test tubes then 2ml of distilled water was added followed by the addition of 0.15 ml of sodium nitrite (5% $NaNO_2$, w/v) and allowed to stand for 6 min. Later 0.15 ml of aluminium trichloride (10% $AlCl_3$) was added and incubated for 6 min, followed by the addition of 2 ml of sodium hydroxide (NaOH, 4% w/v) and volume was made upto the 5ml with distilled water. After 15 min of incubation the mixture turns to pink whose absorbance was measured at 510 nm using a colorimeter. Distilled water was used as blank. The TFC was expressed in mg of catechin equivalents (CE) per gram of extract. All the determinations were carried out six times.

Statisticsl analysis

The results were recorded after repeating the experiments six times. The experimental results were expressed as mean ± standard error (SE) of (6n) measurements. The statistical analysis of the data were carried out using student's t-test and the results were considered significant when $p<0.05$.

RESULTS AND DISCUSSION

The present investigation has been carried out to determine the TPC and TFC present in various solvent extracts of leaf, petiole, stem bark, seed and fruitwall of *O.indicum* (Tables1-2). The quantitative analysis of TPC and TFC of various crude extracts of different organs of *O.indicum* revealed that the methanolic exract of seed and bark contained highest amount of TPC (155.0mgGAE/g), followed by methanolic extract of leaf (151.6mgGAE/g) and fruit wall (143.3mgGAE/g), chloroform extract of fruit wall (131.6mgGAE/g) and petiole (11.53mgGAE/g) where as moderate amounts were recorded in methanolic extract of petiole (84.0mgGAE/g) followed by chloroform extract of seed (80.6mgGAE/g), bark (46.3mgGAE/g) and leaf (41.3mgGAE/g) and least amount of TPC were found in benzene extract of petiole (34.0mgGAE/g) followed by petroleum ether extract of fruit wall (28.3mgGAE/g), benzene extract of seed (24.6mgGAE/g), leaf (17.3mgGAE/g) and bark (15.0mgGAE/g), fruit wall (7.6mgGAE/g), leaf (6.0mgGAE/g), petroleum ether extract of bark (2.6mg GAE/g). Where as TFC was found highest in methanolic extract of bark (207.5mgCE/g) followed by chloroform extract of petiole (103.3mgGAE/g) and fruit wall (66.6mgGAE/g), petroleum ether extract of bark (60.8mgGAE/g), benzene and methanolic extract of seed (56.6mgGAE/g and 52.5mgGAE/g), benzene extract of bark (45.0mgGAE/g), benzene extract of leaf (29.1mgGAE/g), petroleum ether extract of seed (26.6mgGAE/g), benzene extract of petiole (24.1mgGAE/g) petroleum ether extract of petiole (16.6mgGAE/g), chloroform extract of bark (16.6mgGAE/g), methanolic extract of petiole (16.5mgGAE/g), petroleum ether extract of leaf (15.0mgGAE/g), chloroform extract of leaf and seed (14.4mgGAE/g and 14.0mgGAE/g), and less amount of TPC were observed in petroleum ether extract of fruit wall (11.6mgCE/g), methanolic extract of leaf and fruit wall (11.16mgGAE/g and 11.0mgGAE/g), chloroform extract of petiole (10.33mgGAE/g) and least amount of was found in benzene extract of fruit wall(3.0mgGAE/g).(Figs.1-2).

Table 1: Quantification of phenols in different solvent extracts of *Oroxylum indicum*

S.No	Solvent	Fruit Wall	Seed	Leaf	Petiole	Bark
01	Methanol	14.33±0.042	15.5±0.068	15.16±0.061	8.4±0.209	15.5±0.044
02	Chloroform	13.16±0.554	8.06±0.098	4.13±0.042	11.53±0.578	4.63±0.108
03	Benzene	0.76±0.033	2.46±0.098	1.73±0.111	3.4±0.252	1.5±0.068
04	Petroleum ether	2.83±0.484	1.5±0.229	1.53±0.240	0.6±0.126	0.26±0.042

(Values are expressed as mean ± SE mg of Gallic acid equivalent per gram of dry weight(mg GAE/gm) of the extract (six observations of six replicates of each sample extract)).

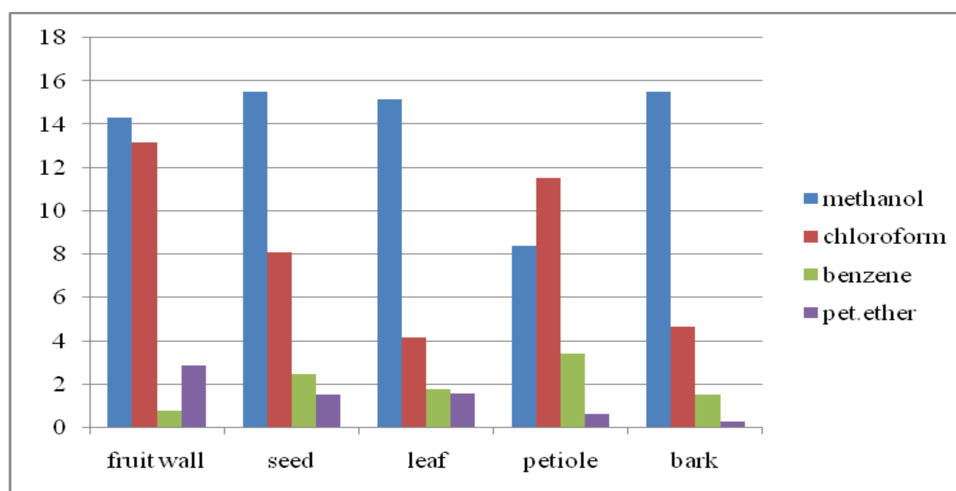

Fig: 1.Showing quantification of phenols in different solvent extracts present in various parts of *O. indicum* in different solvent extracts

Table 2: Quantification of flavonoids in different solvent extracts of *O.indicum*

S.No.	Solvent	Fruit wall	Seed	Leaf	Petiole	Bark
1	methol	1.1	0.525	1.116	1.65	2.075
2	choroform	0.666	1.4	1.44	1.033	1.65
3	benzene	0.3	0.566	0.291	0.241	0.45
4	Petroleum ether	0.116	0.266	0.15	0.166	0.608

(Values are expressed as mean ± SE mg of Catechin equivalent per gram of dry weight(mg CE/gm) of the extract (six observations of six replicates of each sample extract)).

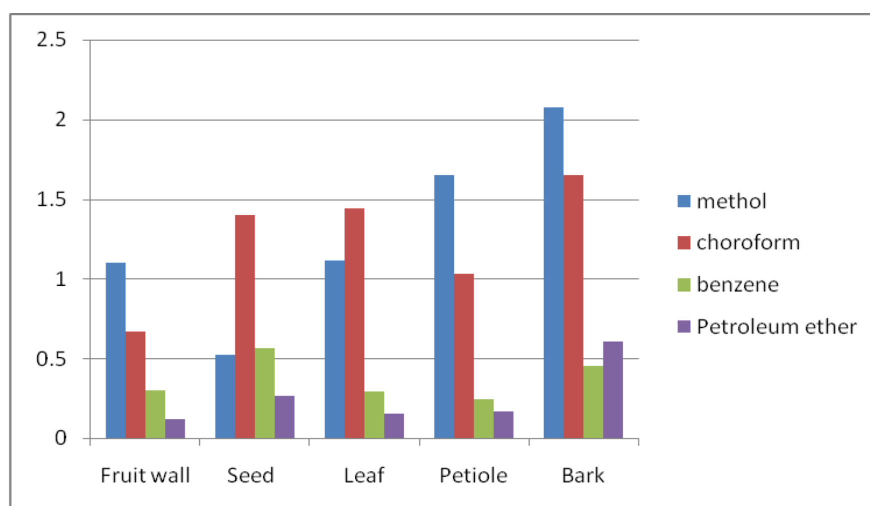

Fig 2:Showing quantification of flavonoids in different solvent extracts present in various parts of *O. indicum* in different solvent extracts

Phytochemicals especially polyphenols constitute a major group of compounds that act as primary antioxidants (Haltano et al.,1989). Relative oxygen species and associated free radicals have been implicated in the etiology of various human diseases including inflammation, metabolic disorders. Cellular aging and atherosclerosis, heart disease, diabetes mellitus, cancer, malaria, rheumatoid arthiritis and HIV/AIDS (Alho and Leinonen, 1999; Odukoya 2005).

More than 4000 phenolic compounds (flavonoids,monophenols and poly phenols) are found in vascular plants. Phenolic compounds such as quercetin, rutin, narigin, catechin, caffeic acid, gallic acid and chlorogenic acid are very important plant constituents (Qusti &al, 2010).

Medicinal plants are known to produce diverse substances possessing antioxidant properties having ability to protect the human body against cellular oxidation. Anti-oxidation are vital substances which possess the ability to protect the body from damage caused by free radicals inducing oxidative stress (Ozsoy et al, 2008). Antioxidant compounds like phenolicacids,polyohenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide of lipid hydroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Subramanian etal.,2011).

Any medicinal plant requires detailed study prior to its use because, the therapeutic efficiency is absolutely dependent on the quality of the plant material used. It has been mentioned that the study of a crude dry of natural origin is useful only to the extent that it contains active principles which have to be identified for the justification its real value (Zaveri and Suvitha jain, 2010).

Conclusion

The results of the present investigation reports the quantitative analysis of total phenolics and flavonoids present in various solvent extracts of different parts of *Oroxylum indicum* .However further investigations are required to isolate and characterize the active constituents from this plant to evaluate their therapeutic role.

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