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Phytochemical and *In-Vitro* Antioxidant Activities of *Melia azedarach* Linn, Catharanthus *Rosea* and Brassica *oleracea L.var.capitata*

KEYWORDS: Melia azedarach Linn, Catharanthus Rosea and Brassica oleracea L.var.capitata, DPPH, Ethanolic extracts and Antioxidant activity.

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ABSTRACT *The present study was to analysis the antioxidant activities of ethanolic leaves extracts of Melia azedarach Linn, Catharanthus Rosea and Brassica oleracea L.var.capitata by DPPH scavenging assay. The phytochemical screening was also carried on the leaves extracts of Melia azedarach, Catharanthus Rosea and Brassica oleracea L.var. capitata revealed the presence of some active ingredients such as Alkaloids, Tannins, Saponins, Phenols, glycosides and flavonoids.The results revealed that the ethanolic extracts of the leaves of Brassica oleracea L.var.capitata had exhibited more antioxidant activity than Catharanthus Rosea and Melia azedarach Linn. While the ethanolic leaves extract of Catharanthus Rosea had exhibited moderate antioxidant activity than other extracts. The high scavenging property of extracts may be due to hydroxyl groups existing in the phenolic compounds.*

INTRODUCTION

Free radicals are the compounds generated from normal body processes and also from environmental pollutions. Within the human body, millions of chemical reactions are occurring constantly .These processes require oxygen. Reactive oxygen spices (ROS), sometimes called active oxygen species, are various from of activated oxygen, which include free radicals such as superoxide ions (O2 -) and hydroxyl radicals (OH-), as well as non-free-radicals species such as hydrogen peroxide (H2O2)[1,2]**.** They tend to attack the healthy cells DNA as well as proteins and fats, causing them to deteriorate. Anti-oxidants are compounds that protect cells against the damaging effects of reactive oxygen specious, such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress has linked to cancer, ageing, atherosclerosis, and ischemia injury, inflammation and neurodegenerative diseases (Parkinson's and Alzheimer's) [3]**.**

Catharanthus Rosea, which is commonly known as 'periwinkle' or '*Vinca rosea*' belongs to family Apocynaceae and is an important source of indole alkaloids, which are present in all plant parts. The physiologically important and antineoplastic alkaloids namely Vincristine and Vinblastine are mainly present in the leaves whereas antihypertensive alkaloids such as ajmalicine, serpentine, and reserpine are reported to be present in the roots[4]. Vincristine and Vinblastine alkaloids are used in the treatment of various types of lymphoma and leukemia [5, 6].

Melia azedarach, the Persian Lilac is popularly known as Maha neem tree and cultivated in all stations. It is a large evergreen tree found throughout India and very similar to Neem. Leaves have been shown to contain nimbinene, meliacin, quercetrin, quercetin-3-0-b-rutinoside, kaempferol- 3-0-b rutinoside, rutin and kaempferol-3-L-rhamno-Dglucoside [7, 8]**.** Hot methanolic etract of *Melia azedarach* leaves contain dipentadecyl ketone, glycerol 1,3-bis-undec-9- enoate 2-dodec-9-enoate and glycerol tris-tridec-9-enoate [9] . The plant is traditionally used for the treatment of leprosy, inflammations, and cardiac disorders. Its fruits extracts possess ovicidal[10] and larvicidal activity[11]. The leaf extracts also possess antiviral [12] and antifertility activity[13] .

Brassica oleracea var. capitata (Cabbage) (Family Brassicaceae) is an excellent source of vitamin C. It also contains significant amounts of glutamine, an amino acid that has anti-inflammatory properties. Cabbage can also be included in dieting programs, as it is a low calorie food.The present study was directed to investigate the hepatoprotective activities of *Brassica oleracea L. var. capitata* against simvastatin induced hepatotoxicity**.** There are increasing evidences that increased consumption of fruits and vegetables and intake of certain non-nutrients that are present in foods reduce the risk of various pathological events such as cancer[14, 15] and cardio- and cerebro-vascular diseases [16]. The vegetables are rich sources of many nutrients and antioxidant vitamins. Therefore, the objective of the present study was to determine the antioxidant activity phytochemical and in-vitro antioxidant activities f *Melia azedarach* Linn, *Catharanthus Rosea(Vinca rosea)* and *Brassica oleracea L.var.capitata (Cabbage)*

MATERIALS and METHOD

Chemicals and Reagents

Folin-Ciocalteu reagent (Merck Pvt. Ltd, India), Sodium chloride (S.D. Fine Chem, India), Sodium carbonet (Merck Pvt. Ltd, India), Catechol (Himedia Lab., India), 2, 2-Diphenyle-2-picryl hydrazyl (DPPH) and Vitamin C are obtained from (Himedia Lab., India).All solutions, including freshly prepared doubled distilled water. Stock solutions of the test extracts were prepared in ethanol. Appropriate blanks were used for individual assays.

Plant collection and identification

The plant material of *Brassica oleracea var. capitata (Cabbage)* ware btain from local market, while *Melia azedarach Linn* and *Vinca rosea* L obtained from Mount Opera Garden, Near Ramoji Film City, Nalgonda Dist. The plant can be identified authenticated by Department of Botany, research office (Botanist), Anwar-ul- loom College of Pharmacy, Hyderabad.

Extraction

The leaves were dried under shade and powdered in a mechanical grinder. The powdered material (250gms) was extracted successively in Ethanol using Soxhlet apparatus at 55°C for 18 h. The extracts was concentrated in vacuo and kept in a vacuum dessicator for complete removal of solvent and weighed.

Phytochemical investigation

The phytochemical studies of leaves of *Brassica oleracea var.*

Volume : 3 | Issue : 7 | July 2013 | ISSN - 2249-555X

capitata (Cabbage), *Melia azedarach Linn* and *Vinca rosea* L were performed for testing the different chemical groups such as alkaloids, tannins, glycosides and saponins etc present in ethanol, petroleum ether and aqueous extracts [17,18].

Antioxidant activity of Melia azedarach,Catharanthus Rosea (Vinca rosea) and Brassica oleracea L. var capitata by using DPPH method

The antioxidant activitties of the *Melia azedarach*, *Vinca rosea* and *Brassica oleracea L. var capitata* on the basis of the scavenging activity of the stable 2, 2- diphenyl-2-picrylhydrazyl (DPPH) free radical was determined according to the method described by Brand-Williams *et al*.(1995)[19] with slight modification. The following concentrations of ethanol extracts were prepared 20µg/mL, 40µg/mL, 60µmg/mL, 80µmg/mL and 10µg/mL. All the solutions were prepared with methanol. 5 ml of each prepared concentration was mixed with 0.5mL of 1mM DPPH solution in methanol. Experiment was done in triplicate. The test tubes were incubated for 30 min at room temperature and the absorbance measured at 517nm. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. Vitamin C (0.1 mg/ml) was used as a standard and the same concentrations were prepared as the test solutions. The different in absorbance between the test and the control (DPPH in methanol) was calculated and expressed as % scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation.

Scavenging effect (%) = (1-As/Ac) ×100

As is the absorbance of the sample at t =0 min.

Ac is the absorbance of the control at t=30 min.

RESULTS

Preliminary phytochemical screening

The ethanolic leaf extracts of *Melia azedarach Linn*, *Vinca rosea* and *Brassica oleracea L.* var *capitata*, Linn, showed the presence of Alkaloids, Tannins, Phenols, Flavonoids, Glycosides and Saponins.

Steroids and Terpenes are absent in *Vinca rosea* and while saponins are absent in *Brassica oleracea L.* var *capitata* (Table 1).

Yields of ethanolic extracts of *Melia azedarach, Vinca rosea and Brassica oleracea L. var capitata*

The variation in yields of ethanolic leaf extracts of *Melia azedarach, Vinca rosea and Brassica oleracea L. var capitata* were 22.13gm, 23.80gm and 27.98gm respectively, shown in Table .2

DPPH free radical scavenging activity of *Melia azedarach, Vinca rosea and Brassica oleracea L. var capitata*

The result shows that the ethanol extract of *Melia azedarach Linn and Brassica oleracea L. var capitata* exhibited the highest radical scavenging activity with 71.42±0.04 and 75.65±0.06 at 100 (µg/ml) concentrations of extracts. While *Vinca rosea* exhibited the moderate radical scavenging activity with 56.43±0.05 at 100(µg/ml) concentration of extract. Fig. 1 and Table.3 Shows the antioxidant activities of different plants extracts.

IC_{50} value

IC50 value is defined as the concentration of substrate that causes 50% loss of the DPPH.The IC_{50} value of *Melia azedarach Linn* , *Vinca rosea* and *Brassica oleracea L.* var *capitata* was calculated as 0.0054µg/ml, 0.0074µg/ml 0.0042 µg/ml respectively. The extracts of *Melia azedarach* and *Brassica oleracea L.* var *capitata* showed highest free radical scavenging activity among with the lowest $IC_{50.}$ While *Vinca rosea* exhibited the moderate radical scavenging activity with the highest IC_{50} as shown in Table 2 and Fig 2.

Table 1. Phytochemical analysis of Ethanolic extracts of *Melia azedarach Linn*, *Vinca rosea* and *Brassica oleracea L.* var *capitata*, Linn.

Sl. No.	Phytochemical Tests	Melia Azedarach	Vinca rosea	Brassica oleracea
1	Alkaloids	+	+	+
2	Steroids	+	-	+
3	Tannins	+	+	+
4	Phenols	+	+	+
5	Flavonoids	+	+	+
6	Glycosides	+	+	+
7	Saponins	+	+	+
8	Terpenes	+	-	-

+= indicates Present of compounds

-=indicates absence of compounds

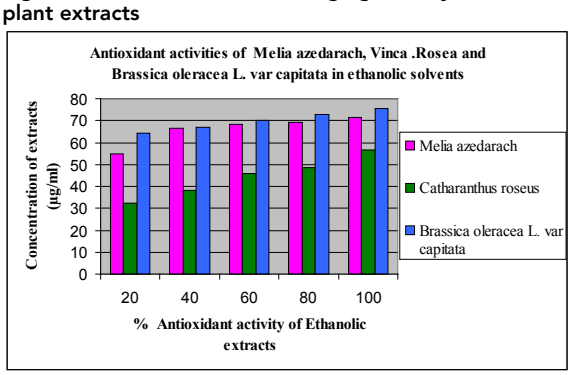
Table-2: Crude extract and IC_{50} Value in Melia azedarach, Catharanthus roseus and Brassica oleracea L. var capitata.

Extracts	Ethanolic Solvent	
	Crude Extracts (gm)	IC 50Value (µg/ml)
Melia azedarach	22.13	0.0054
Catharanthus roseus	23.80	0.0074
Brassica oleracea L. var capitata	27.78	0.0042

Table-3: Antioxidant activities of Melia azedarach, Catharanthus roseus and Brassica oleracea L. var capitata in ethanolic solvents.

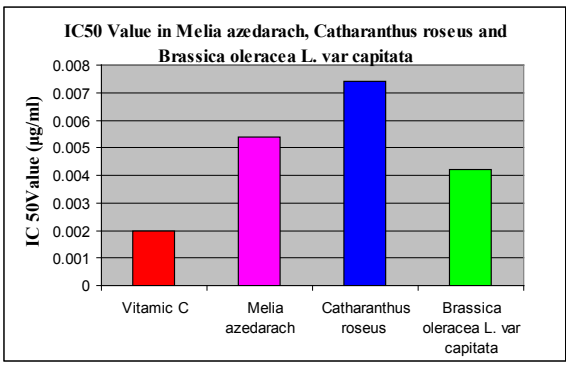
Concentration of extracts (µg/ml)	% Antioxidant activity of Ethanolic extracts		
	Melia azedarach	Vinca rosea	Brassica oleracea L. var capitata
20	54.98±0.05	32.23±0.03	64.31±0.01
40	66.65±0.07	38.26±0.06	67.13±0.02
60	68.38±0.06	45.89±0.03	69.99±0.05
80	69.11±0.03	48.76±0.07	72.76±0.06
100	71.42±0.04	56.43±0.05	75.65±0.06

Fig 1: DPPH free radical scavenging activity of different plant extracts



Volume : 3 | Issue : 7 | July 2013 | ISSN - 2249-555X

Fig 2: IC_{50} Value of different plant extracts compared with Vitamin C.



DISCUSSION

The result of the present study showed that the ethanolic leaf extracts of *Melia azedarach Linn* and *Brassica oleracea L.* var *capitata* exhibited the greatest anti-oxidant activity,while *Vinca rosea* exhibited moderate anti-oxidant activity.The high scavenging property may be due to hydroxyl groups existing in the phenolic compounds. It is reported that phenols are responsible for the variation in the antioxidant activity of the plant [20]. They exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals [21, 22]. It is reported that –OH groups in phenolic compounds are thought have a significant role in antioxidant activity [23]. It is also reported that antioxidant activity of phenolic compounds is mainly due to their redox properties[24]. Methanol and ethanol has been proven as effective solvent to extract phenolic compounds[25]. Ethanol is preferred for the extraction of antioxidant compounds mainly because its lowers toxicity [26].Vegetables contain a wide variety of biologically active, non-nutritive compounds known as phytochemicals. This is often attributed to the antioxidants such as vitamin C, E, carotenoids, lycopenes and flavonoids that prevent free radical damages[27-29].These phytochemicals impart health benefits beyond basic nutrition[30].

CONCLUSION

The result of the present study showed that the extracts of *Melia azedarach Linn* and *Brassica oleracea L.* var *capitata* exhibited the greatest anti-oxidant activity in comparison to *Vinca rosea*.The high scavenging property of *Melia azedarach Linn* and *Brassica oleracea L.* var *capitata* may be due to hydroxyl groups existing in the phenolic compounds chemical structure that can provide the necessary components as a radical scavenger.

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