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(Research Article)

Antioxidant Activity of *Rivea hypocratreiformis, Breynia retusa,Woodfordia fruticosa* used as Traditional Medicine

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ABSTRACT

Presently there have been augmented interests worldwide to recognize antioxidant compounds that are pharmacologically potent along with have near to the ground or no side effects for exploit in anticipatory medicine in addition to the food diligence. As plant life manufacture considerable amount of antioxidants to avoid the oxidative pressure caused by photons moreover oxygen, they correspond to a impending resource of novel compounds in the midst of antioxidant activity. Frequently used therapeutic plant extracts through standardized contented of polyphenols be investigated intended for their antioxidant activity. In the present study, the ethanolic extracts of three plants (Rivea hypocratreiformis, Breynia retusa, Woodfordia fruticosa) and their feasible constituents conscientious intended for its antioxidant assets were compared by reducing power, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity method. The vegetation described include antioxidant ethics that can elucidate and substantiate their use in long-established medicine in the precedent as well as the current. These results recommend that antioxidant activity resolve is of importance for a relative assessment of in vitro antioxidant prospective, although it desires to be combined amid in vivo data for tolerable appraisal of the antioxidant capacity of medicinal plant extracts.

Key Words*:* Antioxidant activity; Reducing powers; DPPH, Indian Medicinal Plants.

INTRODUCTION

Currently, the piece of evidence of detrimental effect of reactive oxygen species on creature health is well-known. The competence of ordinary resistance systems of existing organisms aligned with intemperance creation of these genus decreases as soon as inclined with unenthusiastic ecological factors or aging[1]. The habitual medicine all over the world is at the present time revalue by an wide-ranging activity of study on dissimilar plant variety along with their restorative principles. As flora produce a lot of antioxidants as it have power over the oxidative stress cause by sunbeams also oxygen, they can correspond to a source of new compounds with antioxidant activity[2]. *In vivo*, the reactive oxygen species (ROS) are formed such as superoxide anion, hydroxyl radical moreover hydrogen peroxide, are extremely reactive also potentially damaging transient chemical species. These are incessantly created in the individual body, as they are indispensable for energy supply, detoxification, chemical signalling and immune function.

ROS are synchronized by endogenous superoxide dismutase, glutathione peroxidase plus catalase excluding owing to over-production of reactive species, induce by exposure to peripheral oxidant substances or a malfunction in the defense mechanisms, smash up to cell structures, DNA, lipids or proteins[3] occur which increase risk of additional than 30 different infection processes[4]. The nearly everyone disreputable in the midst of them being neurodegenerative conditions like Alzheimer's disease[5,6], mild cognitive impairment (MCI) and Parkinson's disease (PD). Erstwhile neurodegenerative diseases appreciably related through oxidative stress comprise multiple sclerosis, Creutzfeldt–Jacob disease furthermore meningoencephalitis. Previous diseases consist of highly disabling vascular pathologies like cardiovascular disease (CVD) and cardiac failure[7], alcohol-induced liver disease (ALD)[8] and ulcerative colitis and cancer cause by a complex of different causes, of which RNS/ROS is a component. In Ayurveda, an antique Indian form of medicine with the aim of deals with plants as well as the plant extracts, quite a few herbal formulations encompass been described for liver disorders[9]. In the present study attempt is made to evaluate antioxidant property of few drug extracts of *Rivea hypocratreiformis, Breynia retusa, Woodfordia fruticosa.* Further, these plants were screened for the presence of phyto-chemicals viz. alkaloids, flavonoids, tannins, saponins, glycosides etc.

MATERIALS AND METHODS

Collection

Authentic samples: Various market samples of *Rivea hypocratreiformis, Breynia retusa, and Woodfordia fruticosa* were procured from Chunnilal Attar Ayurvedic Store, Ghat Gate, Jaipur in the month of March, 2010.

Identification

All the samples were authenticated and were given identification number. The identification was as follows:
These samples were authenticated and submitted in Ethnomedicinal Herbarium, Centre of Excellence funded by DST, MGiaS, Jaipur (Rajasthan).

Processing of Plant Materials

During the course of the study each sample was screened for its foreign matter and milled, before use.

Experimental Details:

Present studies were performed on *Rivea hypocratreiformis, Breynia retusa, Woodfordia fruticosa* for the following studies:

1. Phytochemical test of plant extract
2. Antioxidant Potentials of Methanolic extract of plant

PHYTOCHEMICAL SCREENING

Phytochemical screening was performed using standard procedure:

Test for Reducing Sugars (Fehlings Test)

The aqueous ethanol extract (0.5gm in 5 ml of water) was added to boiling fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

Test for Terpenoids (Salkowski Test)

To 0.5 gm each of the extract was added to 2ml of chloroform. Concentrated sulphuric acid (3ml) was carefully added to form a layer. Reddish brown coloration of the interface indicates the presence of terpenoides.

Test for Flavonoides

4 ml of extract solution was treated with 1.5ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated Hydrochloride acid was added and red colour was observed for flavonoids and orange color for flavons.

Test for Tannins

About 0.5 g of the extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for Saponins

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously. And observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for Alkaloids

Alkaloids solutions produce white yellowish precipitate when a few drops of Mayer's reagents are added. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's regent.
The alcoholic extract was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of mayer's reagent. The sample was then observed for the turbidity or yellow precipitation.

EVALUATION OF ANTIOXIDANT ACTIVITY

Preparation of test extracts

All the test plant sample and their adulterants were milled and refluxed in ethanol for 36 h, filtered, concentrated to dryness *in vacuo*. A portion of ethanolic extract was further successively extracted in pet. ether, benzene, chloroform, alcohol and water, concentrated and stored at minimum temperature, until used.

Preparation of DPPH

DPPH (2, 2'-diphenyl-1-picrylhydrazl, $C_{18}H_{12}N_5O_6$; Hi media) 0.8 mg was dissolved in 10 ml methanol to obtain a concentration of 0.08 mg/ml for antioxidative (qualitative and quantitative) assay.

Qualitative Assay

Each successive extract (10 mg) was dissolved in 10 ml of its suitable solvent to get a concentration of 1 mg/ml and from this, 0.25µl was taken with the help of micropipette, applied on silica gel G coated plates. These circular spots were sprayed with DPPH solution, allowed to stand for 30 min. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced, and the changes in colour (from deep- violet to light- yellow on white) were recorded at 517nm on a UV spectrophotometer (Varian Cary PCB 150, Water Peltier System).

Quantitative Assay

A concentration of 1 mg/ml of ethanolic extract of each test sample was prepared to obtain different concentrations (10^2µg to 10^{-3} µg/ ml). Each diluted solution (2.5 ml each) was mixed with DPPH (2.5ml). The samples were kept in the dark for 15 min at room temperature and then the decrease in absorption was measured. Absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured. The UV absorbance was recorded at 517 nm. The experiment was done in triplicate and the average absorption was noted for each concentration. Data were processed using EXCEL and concentration that cause 50% reduction in absorbance (RC_{50}) was calculated. The same procedure was also followed for the standards (Quercetin and Ascorbic acid).

RESULTS AND DISCUSSION

The DPPH radical scavenging activity of three plants is given in the Fig.1. This activity was improved by growing the concentration of the sample extract. DPPH antioxidant assay is based on the capability of 1,1-diphenyl-2-picrylhydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. In the present investigations antioxidant activity of *Rivea hypocratreiformis, Breynia retusa, Woodfordia fruticosa* showed good activity against

the DPPH assay method where the regression line clear cut showed the effectiveness of it as it's have potentials which are comparable to ascorbic acid.

The Phytochemical screening of the plants bare a few differences in the constituent of the tested plants in table 1. The studies showed the presence of of Saponin only in Fire-Flame Bush and Phang plants respectively whereas Phang shows the presence of Alkaloids and Flavonoids. The presence of Tannin were showed in Fire-Flame Bush and Cup-Saucer Plant. The incidence of quercetin in enormous quantity is realistically proportional to the antioxidant activity so it is clearly show that occurrence of flavonoids and this will prove the antioxidant activity and encourage a drug for action of infectious diseases. All the plants exhibit burly antioxidant activity additional or a smaller amount. The amount of flavonoids in the plants is probable to be responsible for the free radical scavenging effects pragmatic.

CONCLUSION

Extracts of three different drugs (*Rivea hypocratreiformis*, *Breynia retusa*, *Woodfordia fruticosa*) were subjected to viewing for their probable antioxidant activity. The two corresponding test systems, specifically DPPH free radical scavenging along with reducing power, were use for the chemical analysis. DPPH is a stable free radical with a distinguishing absorption at 517nm. It was used to swot the radical scavenging effects of extracts. As antioxidants contribute protons to these radicals, the absorbance decreases. The diminish in absorbance is engaged as a evaluate of the extent of radical scavenging. Free radical scavenging capacity of the extracts as well as standard (Ascorbic Acid), measured by DPPH assay was observed. Also the Phytochemical screening of these plants bares a few differences in which *Woodfordia fruticosa* possess a large amount of alkaloids, flavonoids and saponin.

The occurrence of flavonoids will demonstrate the antioxidant activity along with it promotes a drug for treatment of various disease. The incidence of flavonoids in the plants is likely to be responsible for the free radical scavenging effects observed. As a result, this type of study suggest that these plants obtain antioxidant activities which can neutralize the oxidative damage induced by the parasites. This may be one of their mode of action in various disease therapy.

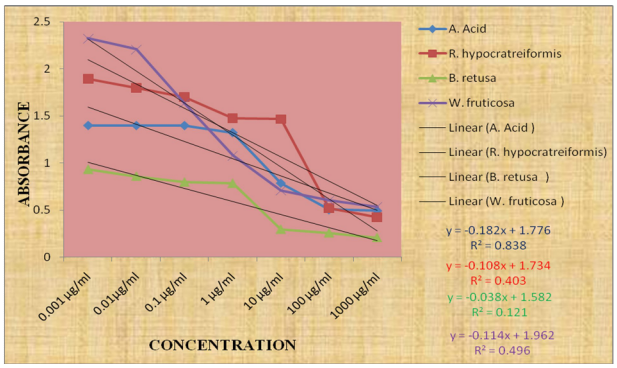

Fig 1: DPPH radical scavenging activity

Table 1: Results of Phytochemical Screening of the plants

Test	Plants		
	Phang	**Cup-Saucer Plant**	**Fire Flame Bush**
Reducing sugars	-	-	-
Saponins	+	-ve	+
Tannins	+	+	-ve
Terpenoides	-	-	-
Flavonoides	-ve	-ve	+
Alkaloides	-ve	-	+

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