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Research Article

PHYTOCHEMICAL SCREENING AND *IN VITRO* FREE RADICAL SCAVENGING ACTIVITY OF *ORTHOSIPHON STAMINEUS* AND *COCCINIA GRANDIS*

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ABSTRACT

Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals." Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Antioxidants are our first line of defence against free radical damage, and are critical for maintaining optimum health and wellbeing. Present study attempts to evaluate the qualitative analysis and free radical scavenging activity of the n Hexane, Chloroform, ethyl acetate and methanol extracts of leaves of *Orthosiphon stamineus* and *Coccinia grandis*. Phytochemical analysis indicated the presence of flavonoids, alkaloids, triterpenes, glycosides, terpenoids, anthraquinones, phytosterol, polyphenol, tannins and sterols. Among all the extracts, methanol extract of both the plants showed highest free radical scavenging activity.

Keywords: Qualitative analysis, *Orthosiphon stamineus* , *Coccinia grandis*, radical scavenging activity, Leaves.

INTRODUCTION

Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction. Their danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane. Cells may function poorly or die if this occurs. To prevent free radical damage the body has a defence system of *antioxidants*.[1,2] Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Antioxidant compounds in plants play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer, kidney disease and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. [3,4] The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. Various antioxidant activity methods have been used to monitor and compare the antioxidant activity of many plants. These analytical methods measure the radical scavenging activity of antioxidants against free radicals like the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, the superoxide anion radical (O2), the hydroxyl radical (OH), the peroxyl radical (ROO) or the nitric oxide radical (NO)

Plants have provided humans with many of their essential needs, including life-saving pharmaceutical agents. Recently the World Health Organization estimated that 80% people worldwide rely on herbal medicines for some aspect. Many developing countries have intensified their efforts in documenting the ethno-medical data and scientific research on medicinal plants. Natural products or natural product derivatives comprised 14 of the top 35 drugs in 2000 based on worldwide sales. There are more than 270,000 higher plants existing on this planet. But only a small portion has been explored phytochemically. So, it is anticipated that plants can provide potential bioactive compounds for the development of new 'leads' to combat various diseases.

Orthosiphon stamineus Benth commonly known as Java tea is a genus of plants in the Lamiaceae family native to Southeast Asia. It is an herbaceous shrub which grows to a height of 1.5 m (4.9 ft). Orthosiphon is a popular garden plant because of its unique flower, which is white and bluish with filaments resembling a cat's whiskers. It is also known as *Ocimum aristatum* BI and *Orthosiphon aristatus* (Blume). In folk medicine, it is used for the treatment of diabetes mellitus and urinary tract infections.[5-9]

Coccinia grandis is a wild cucurbitaceous medicinal plant with many pharmaceutical applications. As an ethnic tribal plant, it has potential therapeutic values as anti diabetic, anti-ulcer, anti-inflammatory, anti-oxidant and anti-tumor properties. The leaf possesses hypoglycemic, antihyperglycemic, antioxidant properties

and is also used to treat infective hepatitis. A perusal of the literature revealed that only fragmentary information was available on these plant species regarding pharmacological activity by any other researchers. Plants are becoming potential source for phytoconstituents with varied pharmacological activities. Identification of such plants of potential use in medicine is of significance.[10-12,19]

So present study is aimed to investigate the phytochemical screening and free radical scavenging activity of *Orthosiphon stamineus* and *Coccinia grandis* and this study would be the leading path way of information for selection of the extract for pharmacological activity and isolation of constituents responsible for the activity**.**

MATERIALS AND METHODS.

Collection and Authentication of plant

The fresh healthy plant leaves of ***Orthosiphon stamineus and Coccinia grandis*** were collected from Irular society, Thandarai, TamilNadu, India during the month of December 2012. The plant was identified and authenticated by Botanical Survey of India, Coimbatore. After authentication, the fresh, healthy plant leaves of *Coccinia grandis* and *Orthosiphon stamineus* were properly dried in shade for 2-3weeks. It was pulverized in a blender, sieved and used for further studies.

Preparation of the Extracts

About 2 kg of air-dried plant material of *Orthosiphon stamineus* and *Coccinia grandis* were extracted in Soxhlet assembly successively with n-hexane, chloroform, ethyl acetate and methanol (order of increasing polarity). Each time before extracting with the next solvent, the powdered material was dried. Each extract was concentrated by using rotary vacuum evaporator. The extract obtained with each solvent was weighed and the percentage yield was calculated in terms of dried weight of the plant material. The colour and consistency of the extract were also noted. All the solvents used for this entire work were of analytical reagent grade.

Qualitative Chemical Tests [13.20.21]

The n-hexane, chloroform, ethyl acetate, methanol extracts of the leaf powder of *Coccinia grandis* and *Orthosiphon stamineus* were subjected to qualitative chemical analysis. Preliminary screening of bioactive compounds namely alkaloids, flavonoids, triterpenes, glycosides, terpenoids, anthraquinones, phytosterol, polyphenol, tannins and sterols.

Estimation of DPPH Radical Scavenging Activity

Free radical scavenging effect was estimated according to the method of Blois [15] as modified by Zhu et.al .[14] Briefly, a 1mM solution of DPPH radical solution in methanol was prepared, and then 1mL of this solution was mixed with different concentrations of methanolic extract; the mixture was then vortexed vigorously and left for 30 min at room temperature in the dark and the absorbance was measured at 517 nm with a spectrophotometer and is calculated.

DPPH Scavenging activity % = [(Control Absorbance – Extract Absorbance)/Control Absorbance] x 100.

For control 1.0 mL of methanol was added to 1mL of 1mM solution of DPPH radical solution and the rest of the procedures remain the same**.**

Superoxide Radical Scavenging Assay

The superoxide radical scavenging activity of the extracts was measured according to the literature method. The reaction mixture containing PMS (0.1 mmol/L), NADH (1 mmol/L), NBT (1 mmol/L) in phosphate buffer (0.1 mol/L, pH 7.4) with different concentrations of the extract was incubated at room temperature for 5 min and the color was read at 560 nm against a blank. The scavenging effect was calculated against the control. [16,18]

Nitric Oxide Radical Inhibition Assay

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interact with oxygen to produce nitrite ions which were measured by Griess reaction. The reaction mixture (3ml) containing sodium nitropruside (10mM) in phosphate buffered saline (PBS) and the varying concentrations of extract and control were incubated in water both at 25^{o}C for 30 minutes. After incubation, 1.5ml of the reaction mixture was removed and 1.5 of Griess reagent was then added. The absorbance of the chromophore formed was evaluated using spectrophotometer at 546nm. [17]

NO Scavenging activity % = [(Control Absorbance – Extract Absorbance)/Control Absorbance] x 100.

For control 1.0 mL of buffer was added to 3mL of 10mM sodium nitroprusside and the rest of the procedures remain the same**.**

RESULTS AND DISCUSSION

Extraction

The percentage yield of successive extractive values for leaves of *Coccinia grandis* and *Orthosiphon stamineus* is tabulated in Table 1.

In the phytochemical analysis different polarity of phytoconstituents were sorted out from the powdered leaves of *Coccinia grandis* and *Orthosiphon stamineus* by using solvents like n-hexane, chloroform, ethyl acetate and methanol by successive extraction using soxhlet apparatus. Successive extractive values revealed the solubility and polarity particulars of the metabolites in the plant. Methanolic extract showed high extractive yield of 8.5 %w/w and 7.9% w/w when compared to other extracts of *Orthosiphon stamineus* and *Coccinia grandis* respectively.

Phytochemical Screening

Qualitative preliminary phytochemical analysis of *Orthosiphon stamineus* and *Coccinia grandis* were performed initially with different chemical reagents to detect the nature of phytoconstituents and their presence in each extract. For *Orthosiphon stamineus* n-Hexane and chloroform extracts showed the presence of steroids. Ethyl acetate extract was found to contain terpenoids, glycosides, carbohydrates, proteins and saponins. Methanolic extract showed the presence of Alkaloids, terpenoids, carbohydrates, flavonoids, phenols, saponins, proteins and glycosides. For *Coccinia grandis* Chloroform extract showed the presence of glycosides and steroids. Ethyl acetate extract was found to contain, Alkaloids, carbohydrates, proteins and saponins. Methanolic extract showed the presence of glycosides, phytosterol, terpenoids, flavonoids, phenols and saponins. The values are presented in table 2.

In-vitro Free Radical Scavenging Activity

The free radical scavenging activity of various extracts of *Coccinia grandis* and *Orthosiphon stamineus* were detected. The methanol extracts of *Coccinia grandis* and *Orthosiphon stamineus* showed the dose dependent free radical scavenging activity in all in vitro assay models. The results indicated that methanol, Chloroform, ethyl acetate and n Hexane extracts, showed good, moderate and poor

activities at various concentrations tested. Among all the extracts, methanol extract showed highest free radical scavenging activity in both the plants. Free radical scavenging activity of various extracts of *Orthosiphon stamineus* and *Coccinia grandis* were presented in Table 3, 4.

Table 1: The percentage yield of successive extracts of the leaves of *Coccinia grandis* (CG) and *Orthosiphon stamineus* (OS)

S. No	Extract	Colour		Physical nature		Percentage yield (w/w)	
		OS	CG	OS	CG	OS	CG
1.	n-Hexane	Green/ Sticky Mass	Green/Sticky mass	Semisolid	Waxy Semisolid	2.0	1.8
2.	Chloroform	Green/ Sticky Mass	Green/Sticky mass	Semisolid	Semisolid	2.0	2.0
3.	Ethyl Acetate	Yellowish green solid mass	Brownish green solid	Solid	Solid	4.5	3.4
4.	Methanol	Brownish green solid mass	Brownish green solid	Solid	Solid	8.5	7.9

Table 2: Phytochemical screening of *Orthosiphon stamineus* (OS) metand *Coccinia grandis* (CG)

S.No	Test	n-Hexane		Chloroform		Ethyl acetate		Methanol	
		OS	CG	OS	CG	OS	CG	OS	CG
1.	Alkaloids	-	-	-	-	-	+	+	-
2.	Carbohydrate	-	-	-	-	+	+	+	-
3.	Glycosides	-	-	-	+	+	-	+	+
4.	Phytosterol	-	-	-	-	-	-	-	+
5.	Fixed oils and Fats	-	-	-	-	-	-	-	-
6.	Tannins	-	-	-	-	-	-	-	-
7.	Phenols	-	-	-	-	-	-	+	+
8.	Proteins	-	-	-	-	+	+	+	-
9.	Gums and Mucilages	-	-	-	-	-	-	-	-
10.	Flavonoids	-	-	-	-	-	-	+	+
11.	Terpenoids	-	-	-	-	+	-	+	+
12.	Steroids	+	-	+	+	-	-	-	-
13.	Saponins	-	-	-	-	+	+	+	+

Note: + ve indicates positive result, whereas – ve indicates negative result

Table 3: Free radical scavenging activity of various extracts of *Orthosiphon stamineus*

S. No	Name of the Extract	Concentration Mcg/ml	Scavenging activity (%)		
			DPPH	Superoxide Radicals	Nitric oxide Radicals
1	n-Hexane	50	12.34±0.12	24.18±0.05	08.14±0.21
2	n-Hexane	100	29.12±0.32	32.01±0.13	12.12±0.13
3	Chloroform	50	31.14±0.24	15.12±0.14	18.23±0.16
4	Chloroform	100	39.32±0.18	26.21±0.24	20.12±0.09
5	Ethyl Acetate	50	23.14±0.21	25.12±0.34	15.12±0.14
6	Ethyl Acetate	100	39.32±0.12	32.13±0.08	26.12±0.08
7	Methanol	50	65.43±0.14	52.18±0.17	45.12±0.18
8	Methanol	100	84.89±0.25	73.33±0.23	55.09±0.12

Table 4: Free radical scavenging activity of various extracts of *Coccinia grandis*

S. No	Name of the Extract	Concentration Mcg/ml	Scavenging activity (%)		
			DPPH	Superoxide Radicals	Nitric oxide Radicals
1	n-Hexane	50	10.21±0.12	11.13±0.14	10.13±0.34
2	n-Hexane	100	18.12±0.09	18.15±0.21	28.12±0.23
3	Chloroform	50	14.12±0.05	08.32±0.24	19.21±0.14
4	Chloroform	100	28.23±0.21	14.21±0.14	22.1±0.21
5	Ethyl Acetate	50	15.42±0.07	24.12±0.09	19.12±0.19
6	Ethyl Acetate	100	18.43±0.06	28.21±0.08	22.32±0.12
7	Methanol	50	42.12±0.34	48.09±0.23	34.12±0.03
8	Methanol	100	62.14±0.12	66.12±0.16	52.14±0.15

CONCLUSION

With the support of phytochemical studies and in-vitro free radical scavenging activities the study concluded that methanol extract is having highest free radical scavenging activity. In vitro free radical scavenging assays indicate that these plant extracts provided significant source of natural antioxidant. Further studies are needed on the isolation and characterization of individual compounds of these plants to elucidate their various antioxidant mechanisms.

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