Research Article **CODEN: AJPCFF** **ISSN: 2321 – 0915**

Asian Journal of Phytomedicine and Clinical Research

Journal home page: www.ajpcrjournal.com

PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIOXIDANT ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *AQUILARIA MALACCENSIS* LEAVES

J. A. Manisha Mascarenhas*[1], E. Akila[2], C. Geetha Priya[3]

[1*]Department of Pharmacology, RR College of Pharmacy, Banglore, India.
[2]Department of Pharmacognosy, RR College of Pharmacy, Banglore, India.
[3]Department of Pharmaceutical Chemistry, RR College of Pharmacy, Banglore, India.

ABSTRACT

The aim of present study was to screen the phytoconstituents and to investigate *in vitro* antioxidant potential of ethanolic leaf extract of the plant *Aquilaria malaccensis* (Thymelaeaceae). The antioxidant activity was assessed by *invitro* methods using DPPH assay, Hydroxyl radical Scavenging assay, Superoxide radical scavenging assay method. And the plant extract shows significant antioxidant property. Preliminary phytochemical investigations were also performed on the leaves of *Aquilaria malaccensis* which shows the presence of saponins, alkaloids, flavonoids, terpenoids, tannins, carbohydrate, glycosides, coumarin, emodins, anthraquinones, resins, phenols.

KEYWORDS

Aquilaria malaccensis, DPPH assay and Flavonoids.

Author for Correspondence:

Manisha Mascarenhas J A,

Department of Pharmacology,

RR College of Pharmacy,

Bangalore, India.

Email: jessicamanisha94@gmail.com

INTRODUCTION

Oxidative stress is that the precursor to oxidative harm. Oxidative stress is that the precursor Oxidative stress happens once there's associate imbalance between the assembly of free radicals and also the body's ability to counteract their damaging effects through neutralization with antioxidants. Oxidative damage is the harm sustained by cells and tissues that are unable to keep up with free radical production and is recognized as an underlying factor in many chronic diseases, including Heart Problems, type 2 diabetes mellitus, and autoimmune disease[1].

Antioxidants are very important molecules that defend the body from harmful effects caused by radical evoked oxidative stress. Proper diet is the best source of free radical scavenging antioxidants to the body of any organism. Spices are the best sources of polyphenolic compounds such as flavoids, flavanoids, phenolic compounds, anthocyanins, phenylpropanoids, anthraquinones which are good antioxidants. Since ancient times herbals are considered as good antioxidants[2].

Aquilaria malaccensis commonly known as Agar Wood occurs in India, Burma, Malaysia, Philippines and Indonesia. It grows Up to 20 - 40 m tall and 60 cm in diameter. Young bark is brown with fine hairs; older bark is sleek and whitish in colour. Wood while not resin is white, lightweight and soft, whereas wood with resin is tough, dark and significant. Leaves alternate, elliptic or lanceo- late, 3-3.5 cm wide and 6-8 cm long with 12-16 pairs of veins. In anthesis a terminal or auxiliary inflorescence. Flowers hermaphroditic, up to five millimetres long, scented and pea green or white.

It is widely used as an astringent, stimulant, tonic herb to relieve spasms, especially of the digestive and respiratory systems, and lowers fevers. In Western, Chinese and Indian medicines the incense is employed against cancer, particularly of the thyroid. In China it's used as a downer for abdominal complaints, asthma, intestinal colic and symptom, associate degreed as an aphrodisiac and carminative[3]. Hence, the current study was designed to screen the phytoconstituents and to evaluate the antioxidant activity of ethanolic leaf extract of the plant *Aquilaria malaccensis.*

MATERIAL AND METHODS

Plant material

The leaves of *A. malaccensis* was collected from the local region of Mangalore in Dakshina Kannada District and authenticated by Taxonomist Mrs. Aparna Upadhyaya. The collected leaves material were cleaned to remove the adhered dust particles and were then shade dried. The dried plant materials were coarsely powdered, weighed and stored in an air tight container till use.

Extraction Procedure

Leaves of *A.malaccensis* (agar wood) type (before and after inoculation (inoculated with fungus) were collected, dried and powdered. Leaf powder (250g) was exhaustively extracted in 1.5 L ethanol solvent for 2 days at room temperature (28 ± 2^0C). The extraction of grounded leaves were further repeated (twice) with ethanol (1L each time). The filtrate from extraction is combined and the excess was evaporated under reduced pressure at 40^0C using a rotary evaporator to give concentrated crude alcoholic extracts, dried in oven at 50^0C. The weights of all the extracts was measured after solvent evaporation and then kept into a glass container prior to use. Extracts were tested for the presence of active principles such as alkaloids, flavonoids, saponins, steroids, terpenoids and tannins.

In Vitro Antioxident Activities

DPPH Radical Scavenging Assay[4]

The antioxidant activity of the ethanolic leaf extract was determined using the DPPH radical scavenging assay method. Freshly prepared DPPH (187µl) was taken in different test tubes protected from sunlight. To this solution added different concentrations (0, 0.25, 0.5, 1, 2, 4, 6, 8, 10µg/ml) of crude leaf extract. The volume made up to 1 ml with ethanol. The tubes were kept in dark and after 20 minutes absorbance was measured at 515nm. Ethanol was used as blank and vitamin C was used as positive control. The concentration of test materials to scavenge 50% DPPH radical (IC_{50} value) was calculated from the graph plotted with % inhibition against concentration. The percent DPPH scavenging effect was calculated by using following equation:

DPPH scavenging effect (%) or Percent inhibition = A0 - A 1 / A0 × 100.

Where A0 was the Absorbance of control reaction and A1 was the Absorbance in presence of test or standard sample.

Superoxide Radical Scavenging Assay[5]

The reaction mixture contained EDTA (0.1M), 0.3mM NaCN, Riboflavin (0.12mM), NBT (1.5 n moles), phosphate buffer (67Mm, pH 7.8) and various concentration (0, 0.25, 0.5, 1, 2, 4, 6, 8, 10µg/ml) of the leaf extract in a final volume of 3ml. The tubes were illuminated under incandescent lamp for 15 minutes. The topical density at 560nm was measured before and after illumination. The inhibition of superoxide radical generation was determined by comparing the absorbance value of the control with that of leaf extract. Vitamin C was used as positive control. The concentration of the extract required to scavenge 50% superoxide anion (IC_{50}) was then calculated.

$$\% \text{ inhibition} = \frac{OD \text{ of control} - OD \text{ of sample}}{OD \text{ of control}} \times 100$$

Hydroxyl Radical Scavenging Activity (Tbars Method)[6]

About 100µg each of deoxyribose, Ferric chloride, EDTA, Ascorbic acid and Hydrogen peroxide were added to test tubes. Then the different concentrations (0, 0.25, 0.5, 1, 2, 4, 6, 8, 10µg/ml) of crude leaf extract was added. The volume was then made up to 1ml with KH_2PO_4 buffer pH 7.4. Incubation was carried out at 37^0C for 1 h; approximately 400µl of the reaction mixture was added to fresh tubes to which added SDS (200µl), acetic acid (1.5ml) and TBA (1.5ml). The volume was made up to 4ml with distilled water and mixed thoroughly. Incubation was carried out at 100^0C for 1 hr. Cooled and added 1ml of distilled water. The mixture was then centrifuged for 15min at 3000rpm and the OD of supernatant was read at 532nm. The concentration of the test materials to scavenge 50% hydroxyl radical (IC_{50} value) was calculated from the graph plotted using % inhibition Vs concentration.

$$\%\text{inhibition} = \frac{OD \text{ of control} - OD \text{ of sample}}{OD \text{ of control}} \times 100$$

RESULTS AND DISCUSSION

Extraction of Plant Material

The dried and powdered leaves *of A. malaccensis* plant were extracted with ethanol. The percentage yield of *A. Malaccensis* leaves before inoculation (BF) and after inoculation (AF)) was found to be 31.20±1.97 and 30.33 ±2.21% respectively (Table No.1)

Preliminary Phytochemical Screening

Phytochemical evaluation was performed with *A. malaccensis* leaves extracts. The extracts showed positive result for saponins, alkaloids, flavonoids, terpenoids, tannins, carbohydrate, glycosides, coumarin, emodins, anthraquinones, resins, phenols. Leucoanthocyanins, proteins and phlobatanins were not shown any positive results (Table No.2).

***In Vitro* Antioxidant Activities**

Effect of Leaf Extracts on DPPH Radical Scavenging Activity

The DPPH radical was effectively scavenged by crude extracts (BF and AF). A dose dependent reduction was observed within the range of concentrations (0-10µg/ml) of the leaf extracts added to the reaction system (Figure No.1). The IC_{50} value of leaf extracts was found to be 4.21 µg/ml and 4.33 µg/ml (Table No.3) respectively. Vitamin C which was used as the positive control exhibited an IC_{50} value of 2.88 µg/ml. (For each value give plus or minus standard deviation).

Effect of Leaf Extracts on Superoxide Radical Scavenging Activity

Superoxide generated in the photo reduction of riboflavin was effectively inhibited by the addition of varying concentration (0-10µg/ml) of leaves extract (Figure No.2). The effect was found to be dose dependent. The concentration of the leaf extracts (BF and AF) needed to scavenge 50% superoxide anion (IC_{50}) was found to be 6.62 and 6.59 µg/ml respectively (Table No.3). Vitamin C which was used as the positive control exhibited an IC_{50} value of 54.17µg/ml. (For each value give plus or minus standard deviation).

Effect of Leaf Extracts on Hydroxyl Radical Scavenging Activity

The leaf extracts shows inhibition of hydroxyl radicals generated by Fe^{3+} /ascorbate / EDTA /H_2O_2 system (Figure No.3). The IC_{50} value of BF extract was found to be 6.03 µg/ml where as that of AF

extract was found to be 5.88 µg/ml (Table No.3). Vitamin C which was used as the positive control exhibited an IC_{50} value of 289.35µg/ml. (For each value give plus or minus standard deviation).

Table No.1: Percentage yields of crude extract of *A. malaccensis* leaves

S.No	Type of leaf	Solvent	Colour of the extract	% yield
1	Before inoculation (BF)	ethanol	Dark green	31.20±1.97
2	After inoculation (AF)			30.33±2.21

Table No.2: Preliminary phytochemical analysis

S.No	Test	Assigned Compound	Result
1	Froth test	Saponins	+ve
2	Emulsion test		
3	Mayer's test	Alkaloids	+ve
4	Dragondorff's test		
5	Wagner's test		
6	Ammonium test	Flavonoids	+ve
7	Shinoda test		
8	Liebermann-Burchard Test	Terpenoids/steroids	+veT
9	Salkowski test		
10	10% ferric chloride test	Tannins	+ve
11	Alkaline reagents test		
12	Lead acetate method		
13	Molisch's test	Carbohydrate	+ve
14	Benedict's test		
15	Fehling's test		
16	Legal's test	Glycosides	+ve
17	Borntrager's test		
18	10% sodium hydroxide Test	Coumarin	+ve
19	Ammonium hydroxide Test	Emodins	+ve
20	Isoamyl alcohol test	Leucoanthocyanins	-ve
21	Million's test	Proteins	-ve
22	Biuret test		
23	Ninhydrin test		
24	Warming test		
25	10% ammonia test	Anthraquinones	+ve
26	Water test	Resins	+ve
28	Ammonium hydroxide Test	Phenols	+ve
29	Lead acetate test		
30	2% hydrochloride test	Phlobatannins	-ve

(+ve - presence of compound, –ve - absence of compound, +T - terpenoids present)

Table No.3: IC_{50} values for *A. malaccensis* leaf extract in various *in vitro* anti-oxidant assay systems

S.No	Compounds	IC_{50} values		
		DPPH radical reduction assay	Superoxide radical scavenging assay	Hydroxyl radical Generation assay
1	BF	4.21µg/ml	6.62 µg/ml	6.03 µg/ml
2	AF	4.33µg/ml	6.59 µg/ml	5.88 µg/ml
3	Standard	2.88µg/ml (Vit. C)	54.17µg/ml	289.35ng/ml (Vit. C)

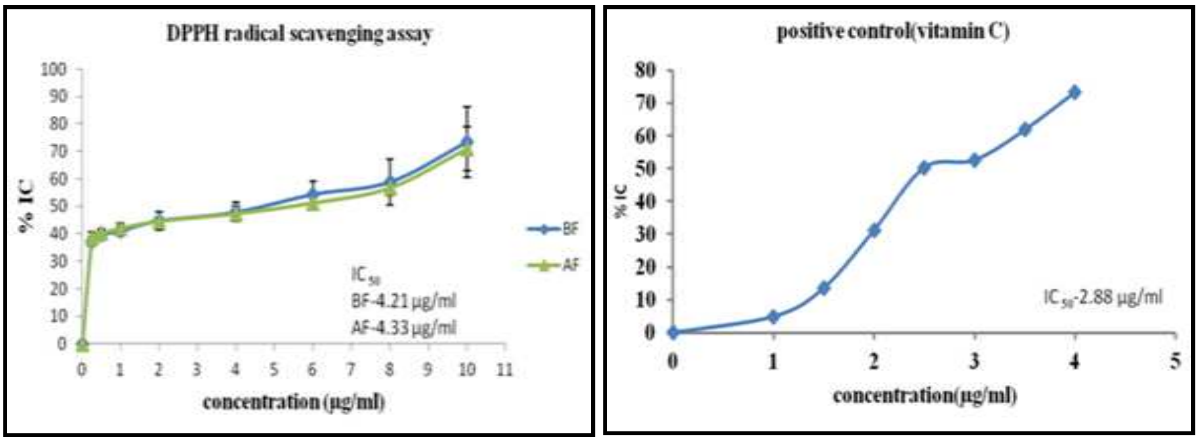

Figure No.1: Effect of leaf extracts on DPPH radical scavenging activity

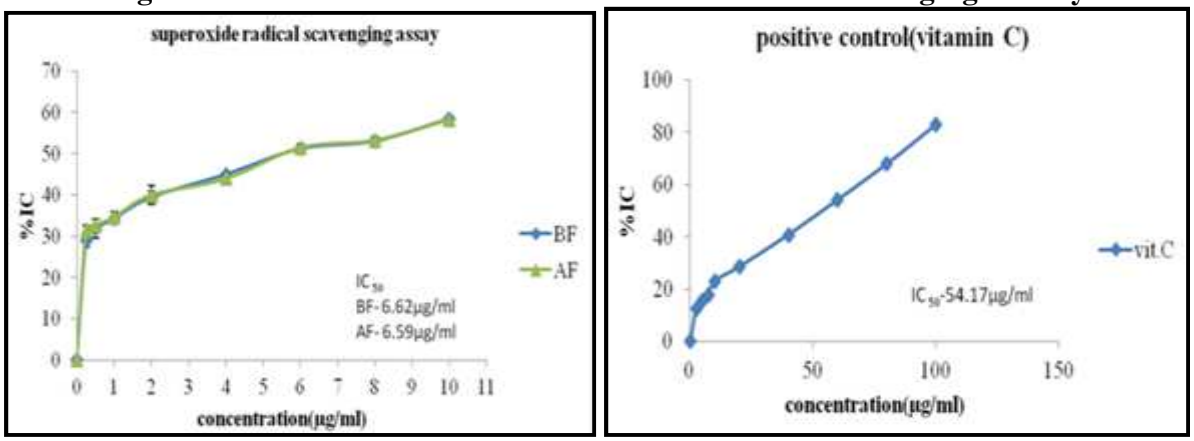

Figure No.2: Effect of leaf extracts on superoxide radical scavenging activity

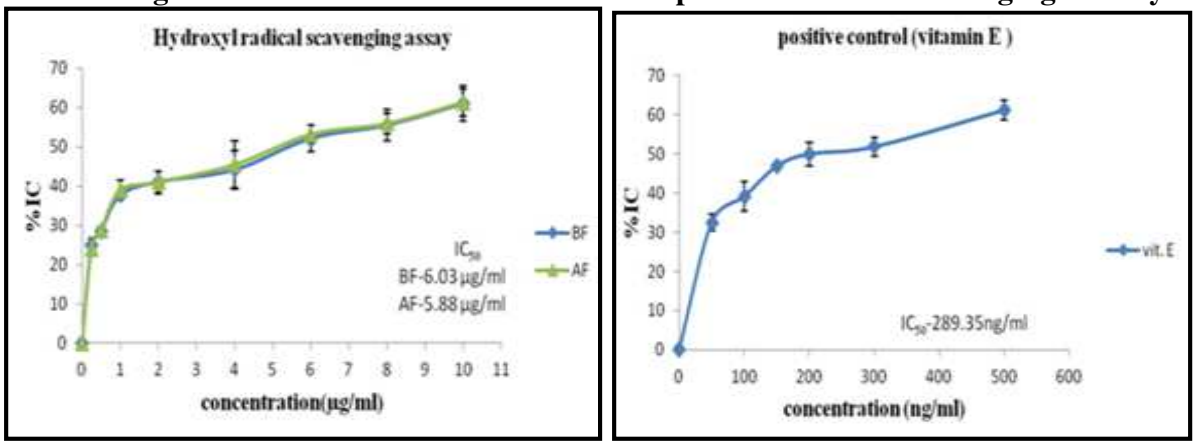

Figure No.3: Effect of leaf extracts on hydroxyl radical scavenging activity

CONCLUSION

In the present work, a medicinally useful plant *Aquilaria malaccensis* was selected. The preliminary phytochemical screening of the leaf extracts indicates the presence of various bioactive secondary metabolites such as alkaloids, anthraquinones, terpenoids, tannins and phenolics. Terpenoids and alkaloid class of compounds are good anti– tumor agents. It is likely that these compounds may be responsible for its cytotoxic property.

In addition the extracts revealed good radical scavenging efficacy. Superoxide anion is one of the most representative free radicals. In cellular oxidation reactions, superoxide radicals have their initial effects magnified because they produce other kinds of cell-damaging free radicals and oxidizing agents such as oxygen radicals and hydroxyl radicals[7]. In the present study the crude extracts showed almost same radical scavenging effect which is more or less similar to vitamin C. In the hydroxyl radical generation assay crude leaf extracts and vitamin C have been found to possess similar efficacy which is highly significant. The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins[8], especially membrane peroxidation. By inhibiting hydroxyl radical mediated damage, the *A. malaccensis* leaf extract find promising role in various degenerative conditions where lipid peroxides and carbonyl are involved. This present study revealed the efficacy of the plant *A. Malaccensis* as antioxidant and thus further investigation can be done.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Department of Pharmacology, RR College of Pharmacy, Banglore, India for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Yang X, Li Y, Li Y, Ren X, Zhang X, Hu D, Gao Y, Xing Y, Shang H. Oxidative stress-mediated atherosclerosis: mechanisms and therapies, *Frontiers in physiology,* 8, 2017, 600.
2. Marka V, Yalavarthy P D. A Study On Antioxidant Activity of Some Commonly Used Spices In India, *International Journal of Life Sciences Biotechnology and Pharma Research,* 2(4), 2013, 145-150.
3. Wang S, Yu Z, Wang C, Wu C, Guo P, Wei J. Chemical constituents and pharmacological activity of agarwood and Aquilaria plants, *Molecules,* 23(2), 2018, 342.
4. Shekhar T C, Anju G. Antioxidant activity by DPPH radical scavenging method of Ageratum conyzoides Linn. Leaves, *American journal of ethnomedicine,* 1(4), 2014, 244-9.
5. Hazra B, Biswas S, Mandal N. Antioxidant and free radical scavenging activity of Spondias pinnata, *BMC complementary and Alternative Medicine,* 8(1), 2008, 63.
6. Bektaşoğlu B, Celik S E, Özyürek M, Güçlü K, Apak R. Novel hydroxyl radical scavenging antioxidant activity assay for water-soluble antioxidants using a modified CUPRAC method, *Biochemical and Biophysical Research Communications,* 345(3), 2006, 1194-200.
7. Halliwell B and Gutteridge J M C. The chemistry of oxygen radicals and other oxygen-derived species. Free Radicals in Biology and Medicine, *New York: Oxford University Press,* 1985, 20-64.
8. Spencer J P, Jenner A, Aruoma O I, Evans P J, Kaur H, Dexter D T, Jenner P, Lees A J, Marsden D C and Haliwell B. Intense oxidative DNA damage promoted by L-dopa and its metabolites: Implications for neurodegenerative disease, *FEBS Letters,* 353(3), 1984, 246-250.

Please cite this article in press as: Manisha Mascarenhas J A *et al.* Phytochemical screening and *in vitro* antioxidant activity of ethanolic leaf extract of *Aquilaria malaccensis* leaves, *Asian Journal of Phytomedicine and Clinical Research,* 6(3), 2018, 99-104.