ISSN No:-2456-2165

Quantification and Antimicrobial activity of Phytochemicals from *Combretum ovalifolium*

G.Shivapriya[1], S. Bhagavathy*[1], P.Arumugam[2]
[1]PG and Research Department of Biochemistry, Mohamed Sathak College of Arts and Science, Sholinganallur, Chennai.
[2] Armats Biotek Training And Research Institute, Chennai.

Abstract:- Natural drugs, and its development from plant based phytochemicals are considered as most important agents for various clinical conditions. Combretum ovalifolium (C.ovalifolium) is one of the phytochemical rich medicinal plants used for variety of diseases. The current research work targets to analyse the qualitative and quantitative existence of bioactive phytochemicals and to check its activity against microbial growth.

Keywords:- *Phytochemicals, Antimocrobial Activity, Combretum Ovalifolium.*

I. INTRODUCTION

Clinical researches focuses on plant based natural products against various pathological conditions, because of its disease curing capacity and its useful medicinal properties **(Umar *et al.*, 2007).** Nowadays many of the researchers focuses on the extraction, purification, and chemotherapeutic activity of bioactive compounds **(Palaksha *et al.*, 2013)**.

New drugs will be developed against various pathological conditions like analgesic, anti arthritic, antipsychotic, and psychotropic agents. **(Pullaiah., 2014; Ramann, 2006)**. The secondary metabolites from medicinal plants act as an excellent and potential bioactive compounds **(Sasco, 2001)**. In future there is a hope on plant discovery, processing, development and screening, for the discovery of new drugs. **(Sonia and Satyanarayana, 2014).**

II. MATERIALS AND METHODS

A. Sources of plant material :

Combretum ovalifolium is collected from Thandalam, Chengalpattu, Tamil Nadu, India. The aerial parts of the plant were selected and dried under shade for the period of 15days, and finely ground by mechanical blender. The chlorophyll content was removed and the powdered material was stored in a container for further use.

B. Preparation of crude extract :

The powdered sample was extracted with methanol with the ratio 1:10 (w/v). The filtrates of the extracts were pooled together and the solvent was removed by rotor evaporator. The dried residues were collected, weighed and stored in a refrigerator at 4^{o}C.

C. Qualitative Phytochemical Screening :

The methanol extract was used for the qualitative analysis to check the availability of alkaloids, glycosides, saponins, phenolics, flavonoids and terpenoids **(Horbone, 1973)**.

D. Quantitative phytochemical analysis :

The methanol extract was used for the quantitative existence of the phenolic content of *C. ovalifolium* leaves by the method of Folin- ciocalteau.

E. In vitro antioxidant assays :

The antioxidant capacity of methanol extract of *C. ovalifolium.* The radical scavenging activity**,** in terms of the $ABTS^{\bullet+}$ was assessed following the procedure described by **Delgado and Andrade, (2005) and Blois, (1958)** and the hydroxyl radical scavenging capacity was evaluated according to the method of **Olabinri and Odedire, 2010)**. Phosphate reduction assay was carried out by the method described by **Prieto *et al* (1999).** The reducing power of methanol extract of aerial parts of *C. ovalifolium* was assessed by the method of **Yen and Chen, (1995)**.

F. Thin layer chromatography :

The active compounds are separated under thin layer chromatography (TLC) using methanol extract. Silica gel coated plates (20X20cm). The methanol extract was spotted and the chromatogram was developed in a mixture of suitable solvent system. The spots were visualized with UV light at 254 nm. The R_f values of the coloured spots were noted and calculated. **(Stahl, 1982)**

G. Antimicrobial Activity :

Effect of methanolic extract against pathogenic microbs such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Proteus vulgaris* were studied. Tetracyclin served as the standard. Fungal strains such as *Aspergillus giganteus*, *Aspergillus niger*, *Mucor*, *Candida albicans*, were used for the antifungal study against fluconazole as the reference standard.

H. Statistical analysis :

The experimental values of triplicates were statistically analysed.

III. RESULTS AND DISCUSSION

A. Qualitative Phytochemical Screening :

Phytochemicals identified in the methanol extract of the selected medicinal plant *C. ovalifolium.* The results pf phytochemical screening shows the existance of alkaloids, terpenoids, steroids, phenolics, flavanoids and glycosides are listed in **Table 1.**

S. No.	Phytochemical Constituents	Result
1.	Alkaloids	+
2.	Terpenoids	+
3.	Steroids	+
4.	Phenolics	+
5.	Flavanoids	+
6.	Glycosides	+
7.	Saponins	-

Table 1:- Phytochemical screening of methanol extract of leaves of *C. ovalifolium*

B. Quantitative phytochemical analysis :

Knowing the valuable phytochemicals presence, the total phenolic content in the methanol extract of *C. ovalifolium* was 0.308 mg/g of gallic acid equivalent. Same way the flavonoid content of methanol extract of leaves of *C. ovalifolium* was 0.08 mg/g of quercetin equivalent.

C. In vitro antioxidant assays :

The radical scavenging activity and antioxidant activity was tested by various assay methods. The antioxidant activities are indicated in **figure 1.** The percentage of inhibition of DPPH radical scavenging activity was 35.11 at 60µg/mL. The IC_{50} value of DPPH radical scavenging activity was 29.25µg/mL concentration. The percentage of inhibition of $ABTS^{\cdot+}$ radical cation scavenging activity was 52.14 at 60µg/mL. The IC_{50} value of $ABTS^{\cdot+}$ radical cation scavenging activity was 43.32µg/mL concentration. The percentage of inhibition of hydroxyl ($\cdot OH$) radical scavenging activity was 13.12 at 100µg/mL. The IC_{50} value of hydroxyl radical scavenging activity was 1.36µg/mL concentration. The reducing power from Fe^{3+} to Fe^{2+} was 0.702 at 60µg/mL. Increase in absorbance of the reaction mixture indicates the reducing power of the extract. The phospho molybdenum reduction capacity was 0.04 at 60µg/mL that indicates the increase in reduction capacity of the extract.

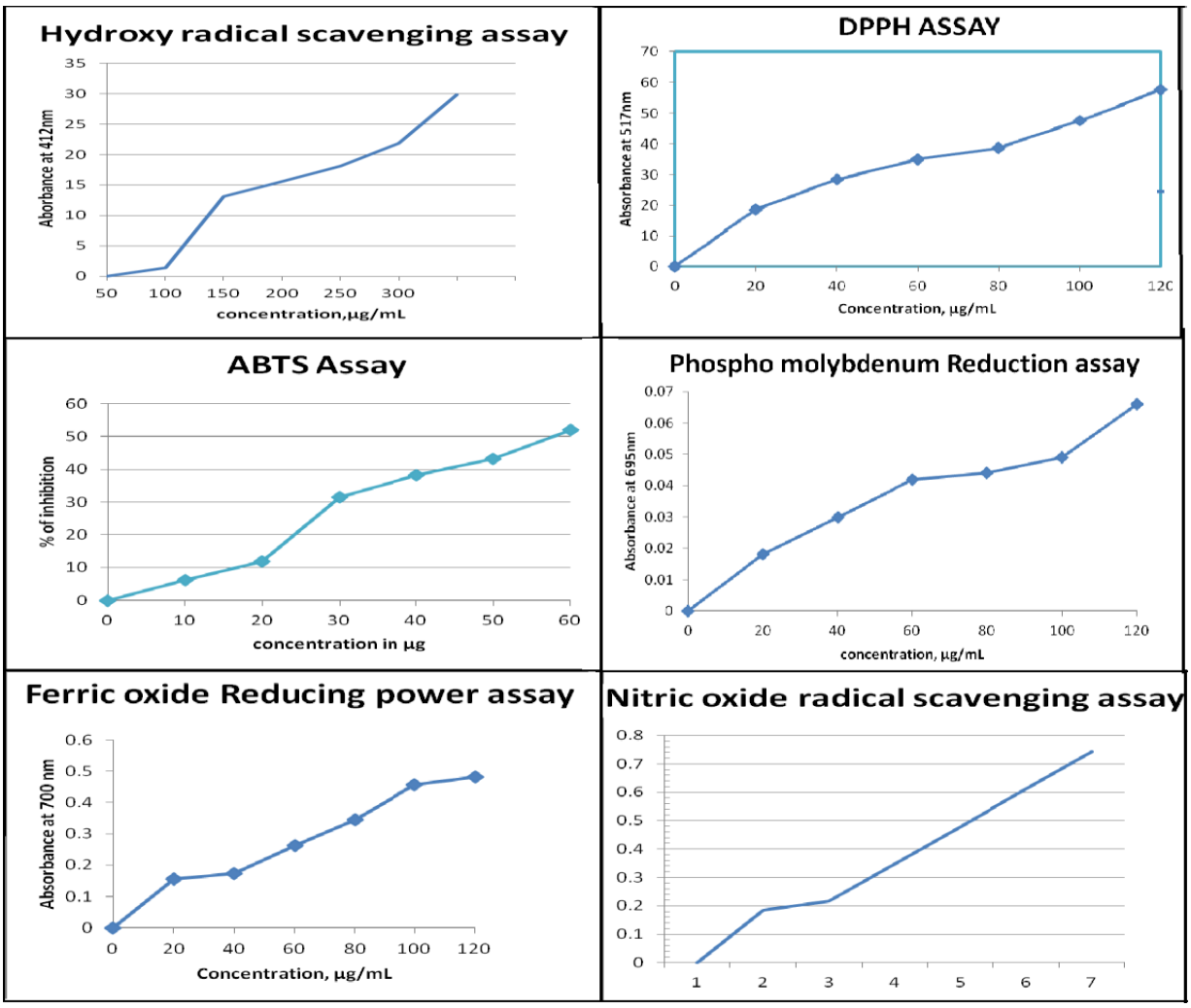

Fig 1:- *In vitro* Antioxidant activity of Methanolic extract of leaves of *C. ovalifolium*

D. Thin layer chromatography :

The bioactive compounds of methanolic extract was separated using thin layer chromatography with the solvent system of Toluene: Ethyl acetate: Methanol with the ratio of 1:0.8:0.2. The compounds separated and confirmed based on R_f values. **(Figure 2).**

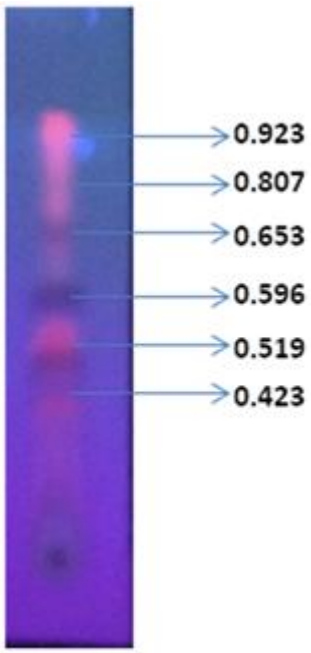

Fig 2:- Thin layer chromatography of Methanolic extract of leaves of *C. ovalifolium*

E. Antimicrobial Activity :

The effect of phytochemicals on the growth inhibition of pathogenic microbial strains reveals the powerful of the medicinal plant. The findings of microbial growth of methanolic extract of leaves of *C. ovalifolium* against the bacterial strains *Escherichia coli, Bacillus subtilis, Klebseilla pneumonia, Staphylococcus aureus, Proteus vulgaris* and fungal strains *Aspergillus gigantea, Aspergillus niger, Mucor, Candida albicans.* The zone of growth inhibition was measured are depicted in **table 2, 3 and figure 3, 4.** The antimicrobial activity of methanol extract of leaves of *C. ovalifolium* showed maximum zone of inhibition of 26 mm against *Escherichia coli.* The antifungal activity of methanol extract of leaves of *C. ovalifolium* showed maximum zone of inhibition of 23mm against *Mucor* and *Aspergillus gigantea.*

S. No.	Organism	Zone of Inhibition (mm)			
		50µl	75µl	100µl	Standard
1.	*E.coli*	23	24	26	13
2.	*B.subtilis*	16	19	20	14
3.	*K.pneumonia*	15	23	24	13
4.	*S.aureus*	18	23	25	13
5.	*P. vulgaris*	18	20	22	14

Table 2:- Anti bacterial activity of Methanolic extract of leaves of *C. ovalifolium*

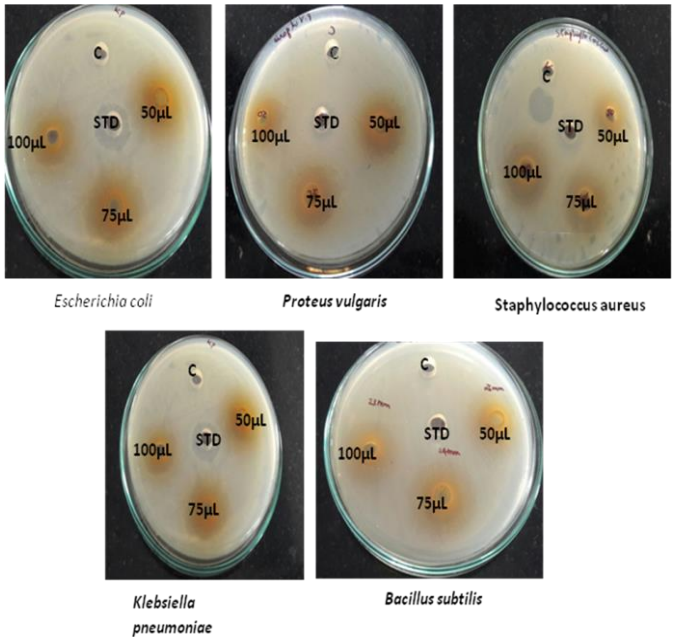

Fig 3 :- Anti bacterial activity of Methanolic extract of leaves of *C. ovalifolium*

S. No.	Organism	Zone of Inhibition (mm)			
		50µl	75µl	100µl	Standard
1.	*A.gigantea*	18	20	23	12
2.	*A.niger*	17	19	20	12
3.	*Mucor*	17	21	23	11
4.	*C.albicans*	17	18	19	12

Table 3 :- Anti fungal activity of Methanolic extract of leaves of *C. ovalifolium*

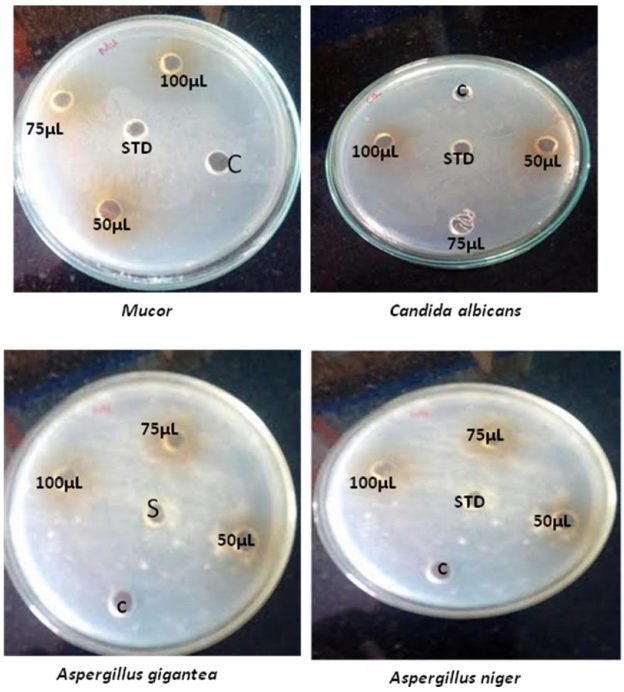

Fig 4:- Anti fungal activity of Methanolic extract of leaves of *C. ovalifolium*

IV. CONCLUSION

The present research findings on the methanol extract of *C. ovalifolium* leaves process phytochemical compounds. The leaves are rich in polyphenols and flavonoids. The observation of the present work suggests that the methanol extract of leaves of *C. ovalifolium* had the significant radical scavenging activity. The phenolics of the methanol extract of leaves of *C. ovalifolium* supports to their scavenging effect. The antifungal activity showed maximum zone of inhibition against *Mucor* and *Aspergillus gigantea.* To summaries the present work the phenolic compounds of the medicinal plant *C. ovalifolium* leaves act as a powerful compounds against infectious agents.

ACKNOWLEDGMENT

The authors wish to acknowledge the management of Mohamed Sathak College of Arts and Science for supporting to carry out the research work.

REFERENCES

[1]. K.J.Umar, L.G.Hassan, S.M.Dangoggo, M.Inuwa M.S.Almustapha, "Nutritional Content of Melochia corchorifolia (Linn.) Leaves". International Journal of Biological Chemistry, 2007; 1 (4): pp.250-255.
[2]. M.N.Palaksha, K.Ravishankar, Girija Sastry, "Evaluation of in vitro antibacterial and anthelmintic activities of *Melochia corchorifolia* plant extracts" International Journal of Biological & Pharmaceutical Research. 2013; 4(8): pp.577-581.
[3]. T.Pullaiah, "Ethnobotany, phytochemistry and pharmacology of *Melochia corchorifolia* L". International Research Journal of Pharmacy, 2014, 5, pp.7.
[4]. N.Raaman, "Phytochemical techniques". New India Publishing Agency, New Delhi, 2006; pp.306.
[5]. A.J.Sasco, "Epidemiology of breast cancer: an environmental disease?" APMIS. 2001, 109(5): pp.321-32.
[6]. Sonia Chauhan, Satyanarayana Labani. "Breast and cervical cancer risk in India: An update. Indian journal of public health", 2014, 58 (1), pp.5-10.
[7]. J.B.Harbone, "Bhytochemical Methods". Chapman and Hall Ltd., London. 1973, pp.49-188.
[8]. M.S.Blois, "Antioxidant determinations by the use of a stable free radical". Nature. 1958; 29: pp.1199-1200.
[9]. C.Delgado-Andrade, F.J.Forales, "Unraveling the contribution of melanoidins to the antioxidant activity of coffee brews". J Agric Food Chem. 2005; 53, pp.1403-1407.
[10]. B.M.Olabinri, O.O.Odedire, M.T.Olaleye, A.S.Adekunle, L.O.Ehigie, P.F.Olabinri, "*In vitro* evaluation of hydroxyl and nitric oxide radical scavenging activities of artemether", Research Journal of Biological Science. 2010; 5, pp.102-105.
[11]. P.Prieto, M.Pineda, M.Anguilar, "Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of Vitamin E". Anal. Biochem. 1999; 269: pp.337-341.
[12]. G.C.Yen, H.Y.Chen, "Antioxidant activity of various tea extracts in relation to their antimutagenicity". *J. Agri.Food Chem.* 1995; 43, pp. 27-32.
[13]. E.Stahl, "Thin Layer Chromatography", 2nd ed., Springer Pvt. Ltd., New Delhi: 2005; pp.85.