In vitro cytotoxic activity of methanolic extract of *Combretum ovalifolium* against MCF7 Cancer cell lines

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***Abstract*: Medicinal plants are important source of potentially useful for the development of novel chemotherapeutic agents. *Combretum ovalifolium* is a common wasteland weed and known for various medicinal properties. The present study investigates the qualitative and quantitative analysis of the major bioactive constituents of medicinally important plant *Combretum ovalifolium* in leaves. The solvents used for the extraction is a methanol. The extract was subjected to qualitative phytochemical screening for the presence of bioactive ingredients and to assess the *in vitro* cytotoxic activity.**

***Keywords*: Phytochemicals, cytotoxic activity, *Combretum ovalifolium*, Cancer cell lines**

1.0 INTRODUCTION

Higher plants have served humankind as sources of medicinal agents since its earliest beginnings. In fact, natural products once served as the source of all drugs. Today, natural products (and their derivatives and analogs) still represent over 50% of all drugs in clinical use, with higher plant-derived natural products representing 25% of the total **(Umar *et al.*, 2007)** On numerous occasions, the folklore records of many different cultures have provided leads to plants with useful medicinal properties. In the past two centuries, the chemical investigation and purification of extracts of plants purported to have medicinal properties, and those used as toxins and hunting poisons in their native habitats, have yielded numerous purified compounds which have proven to be indispensable in the practice of modern medicine **(Palaksha *et al.*, 2013)**.

Plant-Derived Natural Products in Drug Development such as taxol and derivatives and camptothecin and analogs. However, during the course of this screening effort, naturally occurring compounds potentially useful as new drugs for other ailments or conditions (e.g., analgesic, antiarthritic, antipsychotic, and psychotropic agents) were overlooked **(Pullaiah., 2014)**. Thus, since at least 85% of the world's species of higher plants have not been adequately surveyed for potentially useful biological activity, it appears that the plant kingdom has received relatively little attention as a resource of potentially useful bioactive compounds **(Ramann, 2006)**. Because many plant secondary metabolites are genus- or species-specific, the chances are therefore good to excellent that many other plant constituents with potentially useful biological properties remain undiscovered, uninvestigated, and undeveloped **(Sasco, 2001)**. Furthermore, there is the hope that in the future, the process of plant drug discovery and development by way of mass screening will be greatly facilitated and made more efficient by using new automated multiple biological screening methods which are now becoming available and which require only minimal amounts of test samples for evaluation. **(Sonia and Satyanarayana, 2014)**

2.0 MATERIALS AND METHODS

2.1 Collection of plant material: Aerial parts of *Combretum ovalifolium* were collected from Thandalam, Chengalpattu, Tamil Nadu, India. The collected aerial parts were shade dried for 15 days in order to remove chlorophyll content and finely ground by mechanical blender. The powdered material was stored in a container for further use.

2.2 Preparation of crude extract: Finely ground plant material was extracted with methanol in the ratio of 1:10 (w/v) in a conical flask for 72 h. The extract was then filtered using filter paper in a separate container. The above process was repeated for two times with the same residue but using fresh solvent. All the supernatants were collected together and then the solvent was removed by rotor evaporator. Dark gummy mass was obtained, weighed and stored in a refrigerator at 4°C.

2.3 Qualitative Phytochemical Screening: The different qualitative chemical tests were performed to detect alkaloids, glycosides, saponins, phenolics, flavonoids and terpenoids for establishing the profile of given extract for its chemical composition. The tests were done individually for crude methanolic extract **(Horbone, 1973)**.

2.4 Quantitative phytochemical analysis: Total Phenolic content of methanol extract of leaves of *C. ovalifolium* was determined by Folin- ciocalteau reagent method with slight modifications. The total flavanoid content was determined by Aluminium chloride colorimetric method.

2.5 *In vitro* antioxidant assays: The antioxidant activity of methanol extract of aerial parts of *C. ovalifolium* was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhydrazyl (DPPH) free radical. **(Blois, 1958),** in terms of the $ABTS^{\bullet+}$ radical cation scavenging activity following the procedure described by Delgado-Andrade (**Delgado and Andrade, 2005)** and the hydroxyl radical scavenging capacity of methanol extract was evaluated by the method described by **(Olabinri and Odedire, 2010)**. The antioxidant capacity of the methanol extract phosphate reduction assay as decribed by **Prieto *et al* (1999).** The reducing power of methanol extract of aerial parts of *C. ovalifolium* was determined by slightly modified method of **Yen and Chen, (1995)**. Nitric oxide generated from aqueous sodium nitroprusside (SNP) solution interacts with oxygen to produce nitrite ions at physiological pH, which may be quantified and determined according to Griess Illosvoy reaction.

2.6 Thin layer chromatography: Thin layer chromatography (TLC) was carried out for methanol extract of leaves of *C.ovalifolium* on Merck TLC aluminium sheets, silica gel 60 F254 (20 x 20 cm), precoated plates. The extract was spotted at 0.3 mm above from the bottom of the TLC plate. The chromatogram was developed in a mixture of suitable solvent system. The spots were visualized with UV light at 254 nm. The Rf values of the coloured spots were recorded. The ratio in which distinct bands appeared was optimized and Rf values were calculated. **(Stahl, 1982)**

2.7 Cytotoxic activity of *C. ovalifolium on* MCF7 (human breast cancer) cell line: Cell viability was measured with the conventional MTT reduction assay method as described Mossman with slight modification. Briefly, MCF7 cells were seeded at a density of 5×10^3 cells/well in 96-well plates for 24 h, in 200 µL of RPMI with 10% FBS. Then culture supernatant was removed and RPMI containing various concentrations (0.781-100 µg/mL) of test compound were added and incubated for 48 h. After treatment cells were incubated with MTT (10µl, 5 mg/mL) at 37°C for 4 h and then with DMSO at room temperature for 1 h. The plates were read at 595nm on a scanning multi-well Spectrophotometer.

2.8 Statistical analysis: All the experiments were conducted in triplicates and data given in tables were average of the three replicates. All data were reported as mean ± standard deviation of three replicates.

3.0 RESULTS AND DISCUSSION.

3.1 Qualitative Phytochemical Screening:

Direct extraction with methanol was used for the purpose of preliminary screening of *C.gigantea*. The preliminary phytochemical screening of *C. ovalifolium* extract revealed the presence of alkaloids, terpenoids, steroids, phenolics, flavanoids and glycosides are listed in **Table 1.**

Table 1

Phytochemical screening of methanol extract of leaves of *C. ovalifolium*

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

3.2 Quantitative phytochemical analysis:

The phenolic content of methanol extract of leaves of *C. ovalifolium* was 0.308 mg/g and was expressed as gallic acid equivalent. The flavonoid content of methanol extract of leaves of *C. ovalifolium* was 0.08 mg/g and was expressed as quercetin equivalent.

3.3 *In vitro* antioxidant assays:

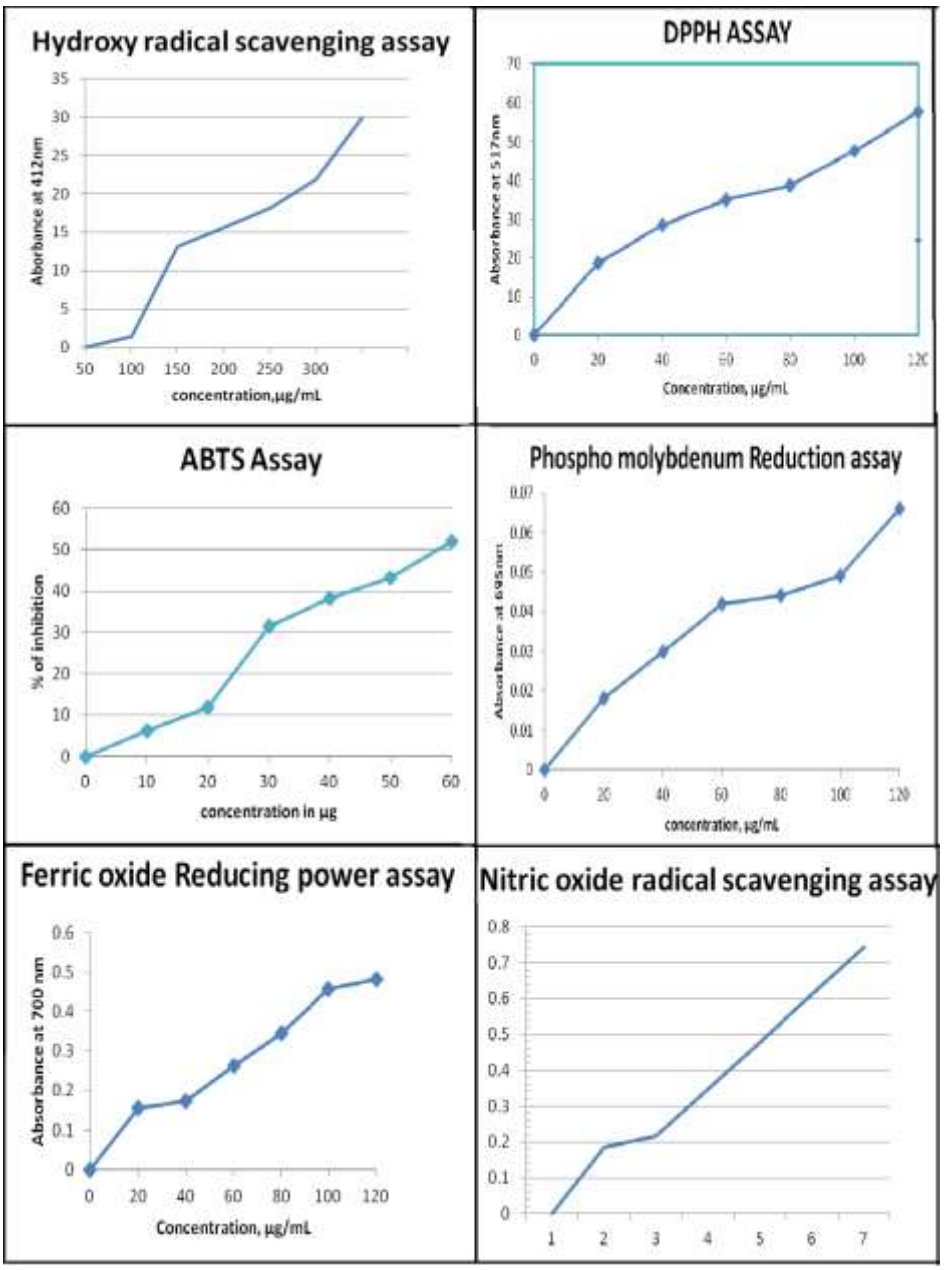
From the dose dependent response curve of antioxidant assay by radical scavenging assay and the other antioxidant assays showed good response in the antioxidant capacity of methanolic extract of *C. ovalifolium.* The antioxidant activity are depicted in **figure 1.**

The percentage of inhibition of DPPH radical scavenging activity was 35.11 at 60 µg/mL. The IC_{50} value of DPPH radical scavenging activity of methanol extract of leaves of *C. ovalifolium.* was 29.25 µg/mLconcentration. 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 of

methanol extract of leaves of *C. ovalifolium* was 43.32µg/mL concentration. The percentage of inhibition of hydroxyl (·OH) radical scavenging activity was 13.12 at 100µg/mL. The IC_{50} value of hydroxyl radical scavenging activity of methanol extract of leaves of *C. ovalifolium* was 1.36 µg/mL concentration. The reducing power of methanol extract of leaves of *C. ovalifolium* from Fe^{3+} to Fe^{2+} was 0.702 at 60 µg/mL. Increase in absorbance of the reaction mixture indicates that the increase in reducing power of the extract. The Phosphomolybdenum reduction capacity of methanol extract of leaves of *C. ovalifolium* was 0.04 at 60 µg/mL. Increase in absorbance of the reaction mixture indicates that the increase in reduction capacity of the extract.

Figure 1

In vitro* Antioxidant activity of Methanolic extract of leaves of *C. ovalifolium

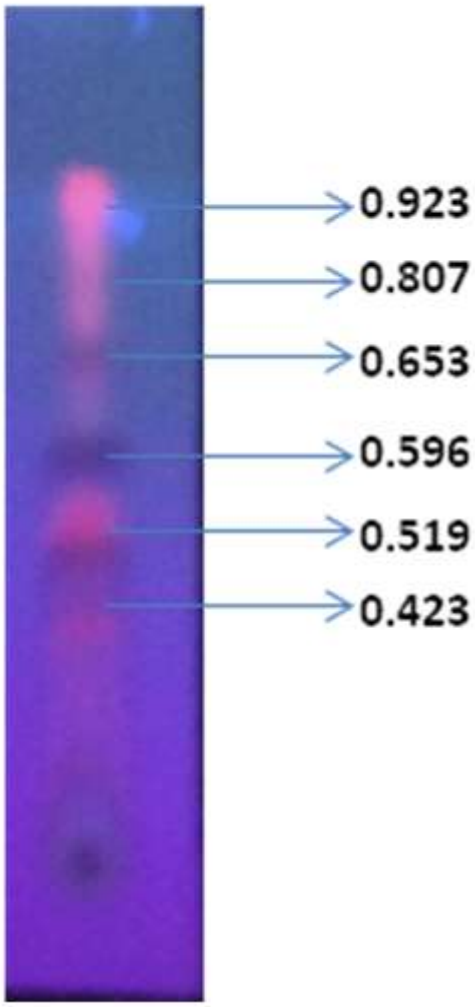


3.4 Thin layer chromatography:

Thin layer chromatography analysis was carried out in the solvent system of Toluene: Ethyl acetate: Methanol with the ratio of 1:0.8:0.2. The compounds of methanolic extract was separated and identified based on Rf values. **(Figure 2).** TLC analysis of methanol extract of leaves of *C. ovalifolium* was carried out with the solvent system of chloroform: methanol : trimethyl amine with the ratio of 1.6:0.3:0.1. The R_f values of the separated compounds were 0.28, 0.31, 0.58.

Figure 2

Thin layer chromatography of Methanolic extract of leaves of *C. ovalifolium*



3.5 Cytotoxic activity of *C. ovalifolium on* MCF7 cell line :

Cytotoxic activity was studied for methanol extract of aerial parts of *C.ovalifolium by* MTT assay method. The morphology of MCF7 cells progressively changed from 12.5 µg/mL to 100 µg/mL concentration of the extract and was compared with control. The maximum cell death was seen 66.84% at 100 µg/mL concentration. **Table 2; Figure 3 & 4**

Table 2

Cytotoxic activity of methanolic leaf extract of *C. ovalifolium*

S.No.	Concentration (µg/mL)	% of Cell death
1	0.781	8.38 ± 1.02
2	1.562	29.26 ± 0.68
3	3.125	34.83 ± 3.33
4	6.25	44.16 ± 3.20
5	12.5	54.14 ± 1.71
6	25	60.79 ± 0.62
7.	50	63.82 ± 1.12

Figure 3

Cytotoxic activity of methanolic leaf extract of *C. ovalifolium* on MCF7 cell line

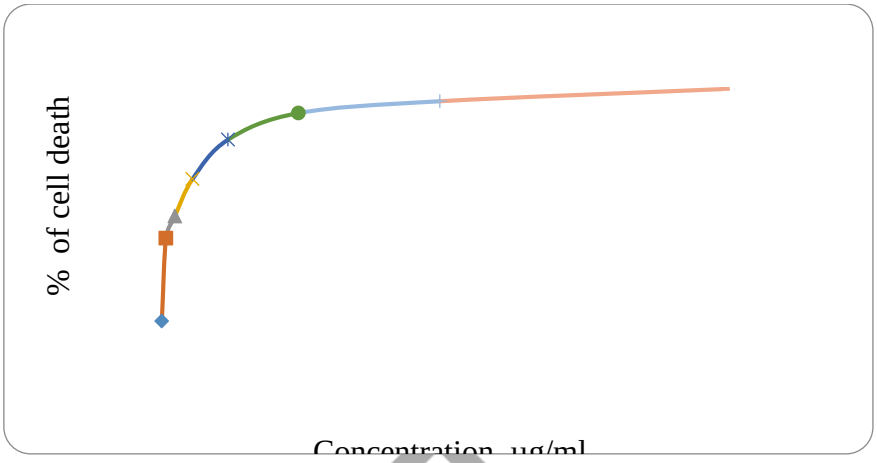
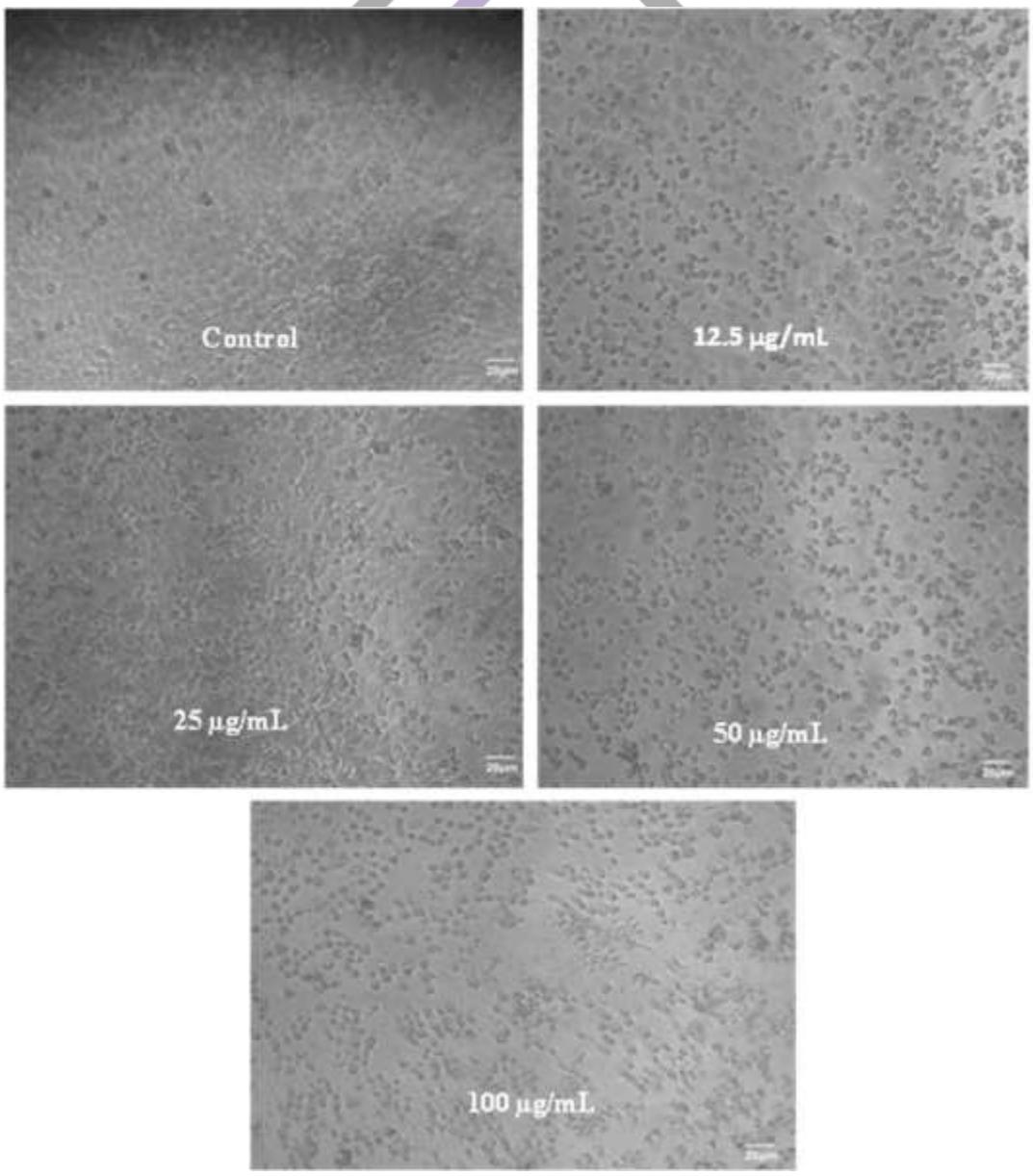


Figure 4

Cytotoxic activity of methanolic leaf extract of *C. ovalifolium* on MCF7 cell line



4.0 CONCLUSION

In the present study the methanol extract of leaves of *C. ovalifolium* rich in phytochemical compounds. The phenolic content of methanol extract of leaves of *C. ovalifolium* was 0.308 mg/g and was expressed as gallic acid equivalent. The flavonoid content of methanol extract of leaves of *C. ovalifolium* was 0.08 mg/g and was expressed as quercetin equivalent. The results of the current study showed that the methanol extract of leaves of *C. ovalifolium* had the significant radical scavenging activity. The phenolic compounds present in the methanol extract of leaves of *C. ovalifolium* correlates to their scavenging effect. These results demonstrate that the antioxidant activities observed can be ascribed both to mechanisms exerted by phenolic compounds and also to synergistic effect of different phyto compounds.

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