QUALITATIVE, QUANTITATIVE AND ANTIOXIDANT STUDIES ON STEM AND LEAF OF *COMBRETUM OVALIFOLIUM*

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ABSTRACT:

The main aim of the present work is to screen antioxidant activity of all the extracts of *Combretum ovalifolium* and quantify the phytochemicals by qualitative and quantitative analysis in different extracts of leaf and stem of *Combretum ovalifolium*. Extraction of dried powder of different parts of *Combretum ovalifolium*, i.e. leaves and stem was carried out in succession with 6 solvents of increasing polarities, viz. hexane, chloroform, ethylacetate, methanol, water and 70%ethanol. Preliminary phytochemical screening was carried out on all the extracts using standard procedures. Radical scavenging activity of different extracts of *Combretum ovalifolium* leaf and stem were studied by DPPH assay. Among these phytocompounds Alkaloids, Carbohydrates, Glycosides, Saponins, Proteins, Fixed Oils and Fats, Phenolics, Flavonoids, Gum and Mucilages, Tannins, Terpenoids, Lignins, Resins, Lactones and Amino Acids were observed in the leaf and stem extracts. Phytosterols, Phlobatannins, Ascorbic Acid, Anthraquinone, Betacyanins, Anthocyanin were absent in both the parts of *Combretum ovalifolium*. The total content of phenolic compounds 1.273 $\pm$0.023 mg/g and 0.7142$\pm$0.564 mg/g, alkaloids 0.468±0.037mg/g and 0.4690±0.370 mg/g, Saponins 0.74±0.055 mg/g and 0.1490±0.283 mg/g, Flavonoids 1.258±0.049 mg/g and 1.6582±0.107 mg/g, terpenoids 2.10±0.112 mg/g and 0.2082±0.382 mg/g were determined in leaf and stem of *Combretum ovalifolium* respectively. Antioxidant results shows that among all the leaf extracts of *Combretum ovalifolium* methanol shows greater activity with IC_{50} value of 4.04mg/ml and similarly, for stem extracts methanol shows highest inhibition with IC_{50} value of 8.02mg/ml.

KEY WORDS: *Combretum ovalifolium*, extraction, phytochemical screening, quantitative estimation, Antioxidant activity.

INTRODUCTION:

Herbal or traditional medicines are relatively safer than synthetic or chemically developed drugs. The knowledge and experience of traditional medicinal practices are very precious for the reason that it comes from thousands of years of trial and errors. Plant-based traditional knowledge has become an organized tool in the search for new sources of drugs and a new chemical entity thus creates the basis of modern medicine and therapeutics.[1] The plant *Combretum ovalifolium* belongs to the family of *Combretaceae* (Rangoo) i.e. creeper family. *Combretum ovalifolium* is a deciduous climber; its leaves are known to have mosquito repellent and insect repellent activity. Traditionally it is utilized for control of insect pest by local farmers and its extract shows reasonable larvicidal properties against vector *Anopheles stephensi*. Similarly leaf extract showed bacterial activity and Stem bark juice of *C. ovalifolium* is administered orally to cure jaundice as well as an antidote for snakebite. A decoction of *C. ovalifolium* leaves is used for curing menstrual problems in Amravati, Maharashtra. It is used as a source of a fiber in South Gujarat, India also fiber from branches are used to preparing cattle, used for rope making [2] and excellent for firewood.[3] The crushed stem is used mostly to clean the vessels as a scrubber. The fiber from the branchlets is used to make rope and leaves are used as fodder.[4] The review of literature reveals that fruit extract shows positive results for the presence of alkaloids and tannins.[5]

The main aim of the present work is to investigate for the phytochemicals present in different solvent extracts of leaf and bark of *C.ovalifolium* by qualitative and quantitative analysis. The phytochemical profile and antioxidant activity of *Combretum Ovalifolium* (*C.ovalifolium*) were first time reported in this article.

MATERIALS AND METHODS:

Sample collection:

The leaves of *C. ovalifolium* were collected from in and around areas in kollidam riverside at Trichy district and used for chemical and preliminary analysis. The taxonomy (GDJ001) identification of the plant was confirmed by the Rapinat Herbarium, St.Joseph's College (Autonomous) Trichy. The taxonomic image and authentication of *C. ovalifolium* was shown in figure-1.

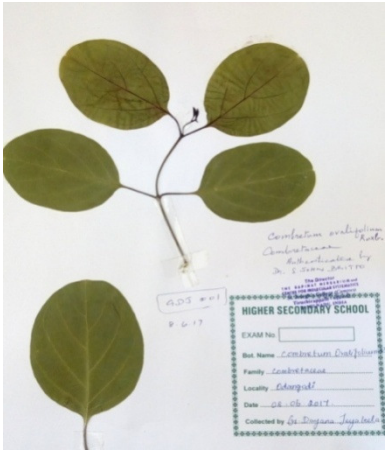

Fig-1: The Taxonomic Image and Authenticated Image of *C. ovalifolium*

Extraction:

Five hundred grams of the shade-dried and powdered *C. ovalifolium* material (leaves & stem powder separately) were packed in a soxhlet apparatus. Initially, hexane is used as an extraction solvent and after completion of extraction; the final extracts were collected and concentrated at below 40° C under reduced pressure using a rotary evaporator. The remaining residual plant material was further extracted with chloroform, ethyl acetate, methanol, water, and 70% ethanol in the same manner as followed for hexane. All the concentrated extracts were subjected to qualitative screening and antioxidant activity. [6]

Qualitative phytochemical screening:

The different qualitative chemical tests can be performed for establishing a profile of twelve extracts for its chemical composition. The following tests were performed on the extracts to detect various phytocompounds present in them.

Detection of alkaloids:

50 mg of a solvent-free extract is stirred with few ml of dilute hydrochloric acid and filtered. The filtrate is tested carefully with various alkaloidal reagents as follows:

Mayer's test:

To a few of filtrate, a drop or two of Mayer's reagent are added by the side of the test tube. A white or creamy precipitate indicates the positive result of alkaloids.

Wagner's test:

To a few ml of filtrate, few drops of Wagner's reagent are added by the side of the test tube. A reddish -brown precipitate confirms the test as positive.

Hager's test:

To a few ml of the filtrate, 1 ml of Hager's reagent (saturated aqueous solution of picric acid) was added. A prominent yellow precipitate indicated the test as positive.

Dragendorff's test:

To a few ml of filtrate, 1ml of Dragendorff's reagent was added. A prominent yellow precipitate indicated the test as positive.

Detection of carbohydrates:

Few mg of the extract was dissolved in 5 ml of water and filtered. The filtrate was subjected to the following tests.

Molisch's test:

To 2 ml of filtrate, two drops of an alcoholic solution of α-naphthol was added, the mixture was shaken well and 1 ml of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand. A violet ring indicated the presence of carbohydrates.

Fehling's test:

One ml of filtrate was boiled on a water bath with 1 ml each of Fehling solutions A and B. A red precipitate indicates the presence of sugars.

Barfoed's test:

To 1 ml of filtrate, 1 ml of Barfoed's reagent was added and heated on a boiling water bath for 2 min. Red precipitate indicates the presence of sugar.

Benedict's test:

To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 min. A characteristic colored precipitate indicated the presence of sugar.

Detection of glycosides:

50 g of an extract was hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate was subjected to the following tests.

Borntrager's test:

To a 2 ml of filtered hydrolysate, 3 ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it. A pink colour indicated the presence of glycosides.

Legal's test:

50 mg of the extract was dissolved in pyridine; sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide. Presence of glycoside is seen by the appearance of pink colour.

Detection of saponins:

The extract was diluted with distilled water and made up to 20 ml. The suspension is shaken in a graduated cylinder for 15min. A two cm layer of foam indicated the presence of saponins.

Detection of proteins:

The extract was dissolved in 10 ml of distilled water and filtered through Whatman No.1 filter paper and the filtrate was subjected to test for proteins and amino acids.

Millon's test:

To 2 ml of filtrate, few drops of Millon's reagent were added. A white precipitate indicated the presence of proteins.

Biuret test:

An aliquot of 2 ml of filtrate was treated with 1 drop of 2% copper sulphate solution, to this 1 ml of ethanol was added, followed by an excess of potassium hydroxide pellets. The pink colour in the ethanolic layer indicated the presence of proteins.

Detection of amino acids:

Ninhydrin test:

Two drops of ninhydrin solution (10mg of ninhydrin in 200ml of acetone) were added to two ml of aqueous filtrate. A characteristic purple colour indicated the presence of amino acids.

Detection of phytosterols:

Libermann-Burchard's Test:

The extract was dissolved in 2 ml acetic anhydride. To this, 2 drops of concentrated sulphuric acid was added slowly along the sides of the test tube. An array of colour changes shows the presence of phytosterols.

Detection of fixed oils and fats:

Spot test:

A small quantity of extract was pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oil.

Saponification test:

A few drops of 0.5 N alcoholic potassium hydroxide solution was added to a small quantity of extract along with a drop of phenolphthalein. The mixture was heated on a water bath for 2 hrs. Formation of soap indicated the presence of fixed oils and fats.

Detection of phenolic compounds:

Ferric chloride test:

Few mg of the extract was dissolved in 5 ml of distilled water. To this, a few drops of neutral 5% ferric chloride solution was added. A dark green colour indicated the presence of phenolic compounds.

Gelatin test:

A small amount of extract was dissolved in 5ml of distilled water and 2ml of 1% solution of gelatin containing 10% sodium chloride is added to it. A white precipitate indicated the presence of phenolic compounds.

Lead acetate test:

Few mg of an extract was dissolved in distilled water and to this; 3 ml of 10 % lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

Detection of flavonoids compounds:

Alkaline reagent test:

An aqueous solution of the extract was treated with 10 % ammonium hydroxide solution. Yellow fluorescence indicated the presence of flavonoids.

Magnesium and hydrochloric acid reduction:

The extract was dissolved in 5 ml of alcohol and few fragments of magnesium ribbon and concentrated hydrochloric acid (drop wise) was added. Change in colour from pink to crimson red infers the presence of flavonol glycoside.

Detection of gum and mucilages:

A small amount of extract was dissolved in 10 ml of distilled water and to this 25 ml of absolute alcohol was added with constant stirring. White or cloudy precipitate indicated the presence of gums and mucilage.

Detection of volatile oil:

In a volatile oil estimation apparatus, 25g of powdered material (crude) was taken and subjected to hydro-distillation. The distillate is collected is graduated tube of the assembly, wherein the aqueous portion automatically separated out from the volatile oil.[7]

Detection of terpenoids:

Salkowshi Test:

The extract was mixed with 2ml of chloroform and concentrated sulphuric acid to form a layer. A reddish-brown coloration at the interface showed the presence of terpenoids.[6]

Detection of phlobatannins:

Formation of red precipitate when an aqueous extract of plant sample was boiled with 1% aqueous hydrochloric acid indicated the presence of Phlobatannins.[8]

Detection of ascorbic acid:

Few mg of the extract was dissolved in water. To that Sodium bicarbonate and ferrous sulphate were added and shake well. The appearance of deep violet colour, disappears on adding 5ml dilute sulphuric acid, indicated the presence of ascorbic acid.

Detection of anthraquinone:

5mg of the extract was hydrolyzed with dilute concentrated sulphuric acid extracted with benzene.1mL of Dilute ammonia was added to it. Rose pink colour indicates the presence of anthraquinones.[6]

Detection of lignins:

Phloroglucinol with Hydrochloric acid was added with the test solution. Formation of pink colour indicated the presence of lignins.[9]

Detection of resins:

One ml of aqueous extract treated with acetic anhydride and concentrated sulphuric acid. Colour changed from orange to yellow shows the presence of resin.

Detection of lactones:

Feigel's test:

The acidified extracts were shaken with solvent ether. To the ether extract with few drops of a saturated alcoholic solution of potassium hydroxide in a porcelain crucible heated over a flame, cooled and appearance of light pink on the addition of 1% $FeCl_3$ to the cooled portion shows the presence of lactones.

Baljel's tests:

The extracts were mixed with a solution of sodium picrate gave yellow-orange color indicated the presence of lactones.[10]

Detection of anthocyanin:

Test-1:

0.5 g of extract was heated with 2M HCl for five minutes at 100°C. the persistence of colour showed the presence of anthocyanin.

Test-2:

0.5 g of extract was treated with 2M NaOH at drop wise. Colour changed to blue showed the presence of anthocyanin.

Detection of betacyanin:

Test-1:

0.5 g of extract was heated with 2M HCl for five minutes at 100°C. Vanishing of colour showed the presence of betacyanin.

Test-2:

0.5 g of extract was treated with 2M NaOH. When the colour changed to yellow it showed the presence of betacyanin.[11]

Quantitative estimation of primary and secondary metabolites:

Determination of total phenols:

The total phenolic contents of the leaf and stem bark of *C. ovalifolium* was estimated by Folin Ciocalteu reagent method.[12] The calibration curve was plotted by mixing 1 ml aliquots of 20, 40, 60, 80 and 100 mg/ml Gallic acid solutions with 0.5ml of Folin Ciocalteu reagent and 0.4 ml of sodium carbonate solution. After 30 min the absorbance was measured at 765 nm. For *C. ovalifolium* leaf and stem extracts, 1 ml of extract was mixed separately with the same reagents, as performed for a standard curve. After 1 h, the absorbance was measured to determine the total phenolic contents in all the extracts separately using the following formula,

$$C = \frac{C1 \times V}{m}$$

Where,

C = Total amount of phenolic content in mg/g, in GAE (Gallic acid equivalent),

C_1 = concentration of Gallic acid established from the calibration curve in mg/ml,

V = volume of extract in ml, and

m = the weight of the plant extract in g

Determination of alkaloids:

10g of the *C. ovalifolium* leaves and stem powder was separately weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and allowed to stand for 4 hr. The whole content was filtered and reduces to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtering then the precipitate was washed with dilute ammonium hydroxide. The total residue is the alkaloid, which was weighed after drying.

Determination of saponins:

10 g of *C. ovalifolium* leaves and stem powder (COL & COS) was separately weighed and soaked in 100 ml of 20% aqueous ethanol. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55^{o}C. The process was repeated till all the saponin content was completely extract, the combined extracts were reduced. The concentrated extract was transferred into a 250 ml separation funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded and this purification process was repeated. 60 ml of n-butanol was added. Finally, the combined n-

butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath and dried in the oven to a constant.[13]

Determination of flavonoids:

10 g of the *C. ovalifolium* (leaves & stem separately) powder was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered and the filtrate was later transferred into a pre-weighed crucible and evaporated into dryness over a water bath. Final residue was considered as total flavonoids content.[8, 10]

Estimation of total terpenoids:

10 g of *C. ovalifolium* leave and stem powder was taken separately and soaked in 100 ml of ethanol for 24 hrs. Then filtered, the filtrate was extracted with petroleum ether. Petroleum ether extract was transferred into a pre-weighed crucible and evaporated into dryness. The weight differences are the total terpenoid contents in plant matter.[14]

Estimation of total chlorophyll content:

100 mg of leaf and stem powder of *C. ovalifolium* have soaked separately in 10 ml of DMSO: acetone mixture (1:1) for overnight incubation (in the dark). The filtrate was filtered and absorbance read at 663 and 645 nm in a spectrophotometer.[15] the total chlorophyll, chlorophyll-a, and chlorophyll-b content were calculated using given standard equations.

$$\text{Chlorophyll} - \text{a (Ca)} = (12.25 \times \text{OD at } 663) - (2.79 \times \text{OD at } 645) \times 10 / (1000 \times \text{Wt})$$

$$\text{Chlorophyll} - \text{b (Ca)} = (21.50 \times \text{OD at } 645) - (5.10 \times \text{OD at } 663) \times 10 / (1000 \times \text{Wt})$$

$$\text{Total Chlorophyll (C)} = (7.15 \times \text{OD at } 663) + (18.71 \times \text{OD at } 645) \times 10 / (1000 \times \text{Wt})$$

Estimation of carotenoids:

10g of the *C. ovalifolium* leaf and stem powder sample was soaked in alcoholic KOH and saponified for about 30 minutes in a shaking water bath at 37°C. After extracting with the alcoholic KOH transferred the extract into a separating funnel containing 10 to 15ml of petroleum ether and shake gently which take up the carotenoid pigments into the petroleum ether layer. Repeat the extraction of the aqueous phase similarly with petroleum ether, until it is colorless. Discard the aqueous layer and preserve the ether layer in an amber bottle. To the petroleum ether extract added a small quantity of sodium sulfate to remove turbidity. The absorbance at 450nm was noted in a spectrophotometer using petroleum ether as a blank. Note the final volume of the petroleum ether extract and diluted if needed by a known dilution factor.

$$\text{Total Carotenoids } (\mu g) = \frac{P \times 4 \times V \times 100}{W}$$

P = Optical density of the sample

V = Volume of the sample

W= Weight of the sample

Estimation of lycopene:

10g of the *C. ovalifolium* leaf and stem powder sample was soaked in alcoholic KOH and saponified for about 30 minutes in a shaking water bath at 37ºC. After extracting with the alcoholic KOH transferred the extract into a separating funnel containing 10 to 15ml of petroleum ether and shake gently which take up the carotenoid pigments into the petroleum ether layer. Repeat the extraction of the aqueous phase similarly with petroleum ether, until it is colorless. Discard the aqueous layer and preserve the ether layer in an amber bottle. To the petroleum ether extract added a small quantity of sodium sulfate to remove turbidity. The absorbance at 503nm was noted in a spectrophotometer using petroleum ether as a blank. Note the final volume of the petroleum ether extract and diluted if needed by a known dilution factor.[16-19]

$$\text{Total Lycopene (mg)} = \frac{3.1206 \times \text{Sample OD} \times \text{Vol. made up} \times \text{Dilution factor} \times 100}{1 \times \text{Weight of the sample} \times 100}$$

Antioxidant Activity of different extracts of *C. ovalifolium* leaf and stem by DPPH assay:

0.3 mM solution of DPPH reagent was prepared by dissolving 11.82 gms of DPPH in 100 mL of ethanol or methanol. Sample stock solution was made by 0.01g in one ml(100mg/ml) and from that different concentration were prepared such as 5, 25, 50, 100, and 200 mg/ml. 1 ml of different concentration of sample solution was mixed with 2 ml of DPPH reagent and allowed to reach room temperature. Thirty minutes later, the absorbance was recorded at 517nm and the percentage of radical scavenging activity i.e anti-oxidant activity was calculated by following standard formulae. Control reading was recorded by one ml of solvent with two ml of DPPH reagent .

$$\boldsymbol{Percentage\ of\ DPPH\ Scavenged} = \frac{\boldsymbol{Ab\ of\ Control - Ab\ of\ Test}}{\boldsymbol{Ab\ of\ Control}} \times \mathbf{100}$$

Where, **Ab of control** - Control Absorbance, **Ab of test**- Test solution Absorbance

The IC_{50} values were calculated by linear regression of plots, where the abscissa represented the concentration of the tested sample and the ordinate the average percent of radical scavenging activity.[20-23]

Statistical Analysis

All the quantitative analyses were performed in triplicate and the results were statistically analyzed and expressed as mean (n=3) ± standard deviation (SD).

RESULTS AND DISCUSSION:

Extraction:

Various extracts of leaf and stem of *C. ovalifolium*'s yield and its percentage are shown in table-1. The graphic representation figure-1 explains that Methanolic extract of COL has a

maximum yield (7.37%) when compared to all the extracts followed by 70% ethanol extract (6.64%) of COL. Of all the crude solvent extracts obtained from the leaf and stem, methanolic extract of leaf (COL-methanol) shows high yield.

Table-1: Yield and percentage of various extracts of *C. ovalifolium*

S.No	solvents used for extraction	the yield of the COL extracts (g)	Yield in percentage	the yield of the COS extracts (g)	Yield in percentage
1	Hexane	2.523±0.7341	5.04%	2.328±0.7204	4.66%
2	Chloroform	3.127±0.8703	6.25%	2.046±0.3986	4.09%
3	Ethyl Acetate	2.840±0.2405	5.68%	1.415±0.4102	2.83%
4	Methanol	3.685±0.6241	7.37%	3.177±1.2005	6.35%
5	Water	2.912±1.1310	5.82%	2.448±0.4755	4.90%
6	70% Ethanol	3.321±1.4285	6.64%	2.136±0.2983	4.27%

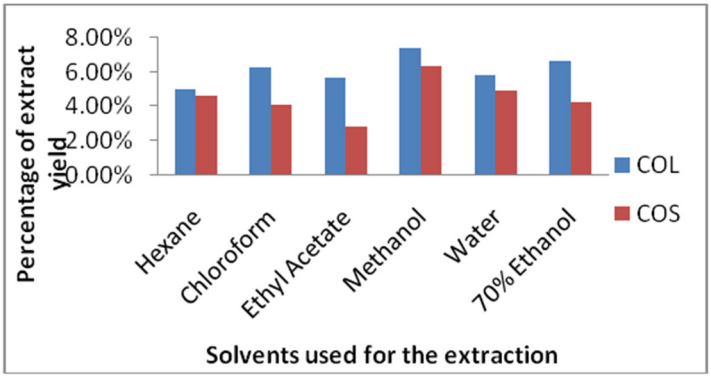

Fig-1: Comparison of Yields Percentage for six Extracts

Phytochemical screening results:

The screening results are compared for all the solvent extracts of COL and COS and their comparison chart is shown in figure-2. The table (2 &3) and the graph (2) reveals that good phytochemical profile is seen for ethyl acetate and methanol fractions of COL while methanol and ethanolic extracts for COS compared to other solvents.

Table – 2: Result of the Qualitative Analysis of *C. ovalifolium* leaf extracts

S.NO	NAME OF THE PHYTOCHEMICALS	S1	S2	S3	S4	S5	S6
1	**Alkaloids:** a) Mayer's Test	-	-	+	+	+	-
	b) Wagner's Test	+	+	+	+	+	+
	c) Hager's Test	-	-	-	-	-	-
	d) Dragendorff's Test	-	+	+	+	+	+

2	**Carbohydrates:** a) Molisch's Test	-	-	+	+	-	+
	b) Fehling's Test	-	-	+	+	-	-
	c) Barfoed's Test	-	-	+	-	-	-
	d) Benedict's Test	-	-	+	+	-	+
3	**Glycosides:** a) Borntrager's Test	-	-	+	+	-	-
	b) Legal's Test	-	+	-	+	-	-
4	**Saponins:**	+	+	-	+	-	-
5	**Proteins:** a) Millon's Test	+	-	+	+	-	-
	b) Biuret Test	-	+	+	+	-	-
6	**Amino Acids:** a) Ninhydrin Test	-	-	-	-	-	-
7	**Phytosterols**	-	-	-	-	-	-
8	**Fixed Oils and Fats:** a) Spot Test	-	-	+	-	-	-
	b) Saponification Test	+	-	-	-	-	-
9	**Phenolics:** a) Ferric chloride Test	-	-	-	-	+	+
	b) Gelatin Test	-	-	-	-	-	-
	c) Lead Acetate Test	-	-	-	-	-	+
10	**Flavonoids:** a) Alkaline Reagent Test	-	+	-	-	+	+
	b) Mg and HCL Reduction	-	-	-	-	+	+
11	**Gum and Mucilages:**	+	-	-	-	-	-
12	**Tannins:** a) Test-1	-	+	+	-	-	-
	b) Test-2	-	-	+	-	-	-
13	**Terpenoids:** a) Salkowshi test:	-	-	+	+	-	+
14	**Phlobatannins:**	-	-	-	-	-	-
15	**Ascorbic Acid:**	-	-	-	-	-	+
16	**Anthraquinone:**	-	-	-	-	-	-
17	**Lignins:**	-	-	-	+	-	-
18	**Resins:**	-	-	-	-	-	-
19	**Lactones:**	-	-	+	+	+	-
20	**Betacyanins:**	-	-	-	-	-	+
21	**Anthocyanins:**	-	-	-	-	-	-

Table – 3: Result of the Qualitative Analysis of *C. ovalifolium* stem extracts

S.NO	NAME OF THE PHYTOCHEMICALS	S1	S2	S3	S4	S5	S6
1	**Alkaloids:**						
	e) Mayer's Test	-	-	-	+	+	+
	f) Wagner's Test	-	-	+	+	+	+
	g) Hager's Test	-	-	-	-	-	-
	h) Dragendorff's Test	-	-	+	+	+	+
2	**Carbohydrates:**						
	e) Molisch's Test	-	-	-	+	-	+
	f) Fehling's Test	-	-	-	+	-	+
	g) Barfoed's Test	-	-	-	+	-	-
	h) Benedict's Test	-	-	-	+	-	+
3	**Glycosides:**						
	c) Borntrager's Test	-	-	-	+	-	+
	d) Legal's Test	-	-	+	-	+	+
4	**Saponins:**	+	-	+	+	-	+
5	**Proteins:**						
	c) Millon's Test	+	-	-	+	-	+
	d) Biuret Test	-	+	+	+	-	+
6	**Amino Acids:**						
	b) Ninhydrin Test	-	+	+	-	-	-
7	**Phytosterols**	-	-	-	-	-	-
8	**Fixed Oils and Fats:**						
	c) Spot Test	-	-	-	+	-	+
	d) Saponification Test	+	-	-	-	-	-
9	**Phenolics:**						
	d) Ferric chloride Test	-	-	-	+	-	+
	e) Gelatin Test	-	-	+	+	-	-
	f) Lead Acetate Test	-	-	-	+	-	+
10	**Flavonoids:**						
	c) Alkaline Reagent Test	-	-	+	+	-	+
	d) Mg and HCL Reduction	-	-	-	+	+	-
11	**Gum and Mucilages:**	+	-	-	-	-	-

12	**Tannins:** c) Test-1	-	-	+	+	-	-
	d) Test-2	-	+	-	+	-	-
13	**Terpenoids:** b) Salkowshi test:	-	-	+	-	-	+
14	**Phlobatannins:**	-	-	-	-	-	-
15	**Ascorbic Acid:**	-	-	-	-	-	-
16	**Anthraquinone:**	-	-	-	-	-	-
17	**Lignins:**	-	-	-	-	-	+
18	**Resins:**	-	-	-	-	-	-
19	**Lactones:**	-	-	-	+	+	+
20	**Betacyanins:**	-	-	-	-	-	-
21	**Anthocyanins:**	-	-	-	-	-	-

(+) - indicates the presence of phytochemicals

(-) -indicates Absence of phytochemicals

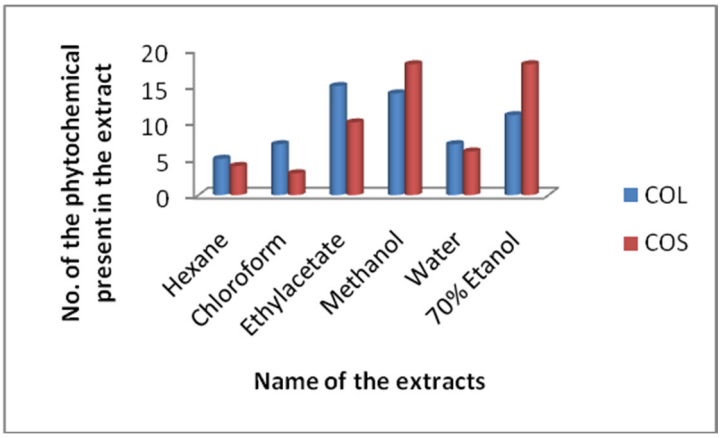

Figure-2: The comparison of qualitative results of various extracts of *C. ovalifolium* leaf and stem

Quantitative analysis result:

Phytochemicals such as primary and secondary metabolites which are present in the leaf and stem of the *C. ovalifolium* are estimated quantitatively and the results are displayed in table-4. The total phenolic content is determined by using gallic acid as a standard, its standard curve shown in graph-3. The histogram figure-4 and table-4 shows that COL contains high percentage of flavonoids, phenolics compared to other metabolites.

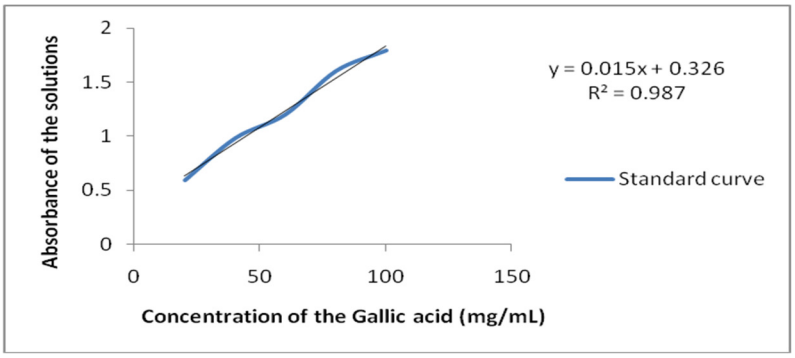

Figure-3: Standard curve for Phenols using Gallic acid as a standard

Table- 4: Quantitative Estimation of Phytochemicals in leaf and the stem portion of *C. ovalifolium*

S.No	Name of the Phytochemicals	The total quantity of phytochemicals in COL (g)	percentage	The total quantity of phytochemicals in COS (g)	Percentage
1	Phenols	1.273±0.023	12.73%	0.7142±0.564	14.28%
2	Alkaloids	0.468±0.037	4.68%	0.4690±0.370	4.71%
3	Saponins	0.74±0.055	7.4%	0.1490±0.283	1.49%
4	Flavonoids	1.258±0.049	12.58%	1.6582±0.107	33.16%
5	Terpenoids	2.10±0.112	21%	0.2082±0.382	2.08%
6	Chlorophyll-a	0.0946±0.005	0.946%	0.004640.741	0.46%
7	Chlorophyll-b	0.164±0.017	1.64%	0.00742±0.179	0.74%
8	Total Chlorophylls	0.258±0.042	2.58%	0.01206±0.952	1.21%
9	Carotenoids	0.350±0.009	3.5%	0.3786±0.751	7.57%
10	Lycopene	0.0064±0.541	0.064%	0.01485±0.604	0.297%

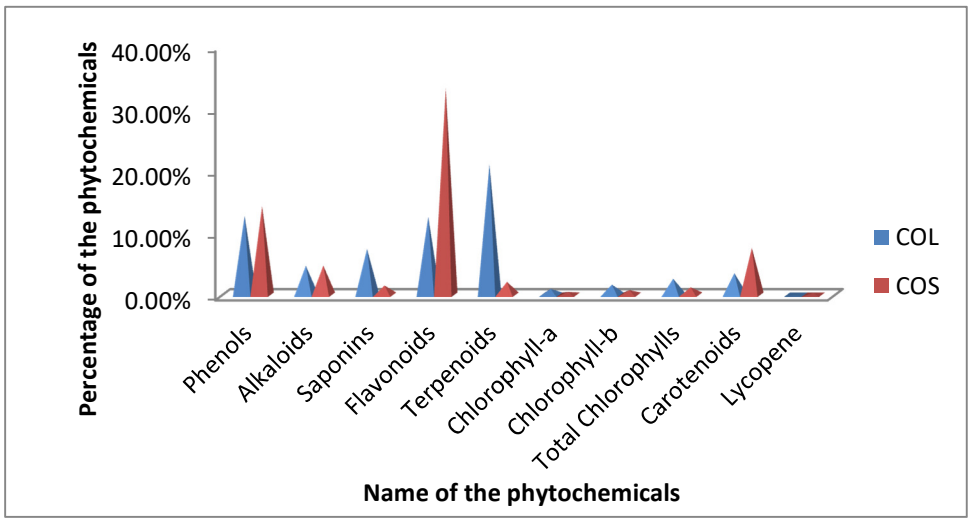

Figure-4: Comparative results of Quantitative estimation on *C. ovalifolium* leaf and stem

Antioxidant activity of different extracts of *C. ovalifolium* leaf and stem:

The results of antioxidant activities of various solvent extracts of *C. ovalifolium* leaf and stem were shown table-5&6 and the comparison of IC_{50} values of *C. ovalifolium* leaf and stem were shown in figure-5. From the table 5&6 the results reveals that the inhibition efficiencies were increases when increase the concentration of extracts and at particular concentration the inhibition efficiency gets decreased due to deficiency of DPPH for further reduction. From the figure -5 the lowest IC_{50} value of *C. ovalifolium* leaf extracts is methanol (4.04 mg/ml) followed by 70% ethanol (37.31mg/ml) and similarly for *C. ovalifolium* stem extracts methanol (8.02 mg/ml)shows lowest IC_{50} value followed by 70% ethanol with 34.43 µg/ml concentration.

Table-5: Antioxidant activity of the different extract of *C. ovalifolium* leaf

S.NO	COL- Extracts	Sample Concentration (mg/ml) with the Respective % of Inhbition					IC_{50} Value mg/ml
		5	25	50	100	200	
1	Hexane	1.25	7.73	10.60	11.11	21.63	469.68
2	Chloroform	2.58	12.73	25.09	34.95	44.74	253.73
3	Ethylacetate	7.51	11.55	35.47	68.36	82.05	72.09
4	Methanol	56.38	91.01	93.27	93.38	92.14	4.04
5	Water	4.60	9.272	37.69	73.22	90.37	67.32
6	70% Ethanol	18.49	33.78	66.73	95.76	95.03	37.31
7	Ascorbic acid (Standard)	9.08	21.75	48.29	80.31	98.12	51.72

Table-6: Antioxidant activity of the different extract of *C. ovalifolium* stem

S.NO	COS-Extracts	Sample Concentration (mg/ml) with the Respective % of Inhbition					IC_{50} Value mg/ml
		5	25	50	100	200	
1	Hexane	1.40	2.50	18.32	23.33	32.16	402.04
2	Chloroform	1.62	12.29	32.89	48.12	63.28	112.40
3	Ethylacetate	23.19	43.61	78.66	88.99	94.96	29.56
4	Methanol	44.53	80.78	92.51	93.16	91.98	8.02
5	Water	9.85	22.40	44.21	59.88	93.86	68.47
6	70% Ethanol	16.37	36.45	72.37	93.44	92.98	34.43
7	Ascorbic acid (Standard)	9.08	21.75	48.29	80.31	98.12	51.72

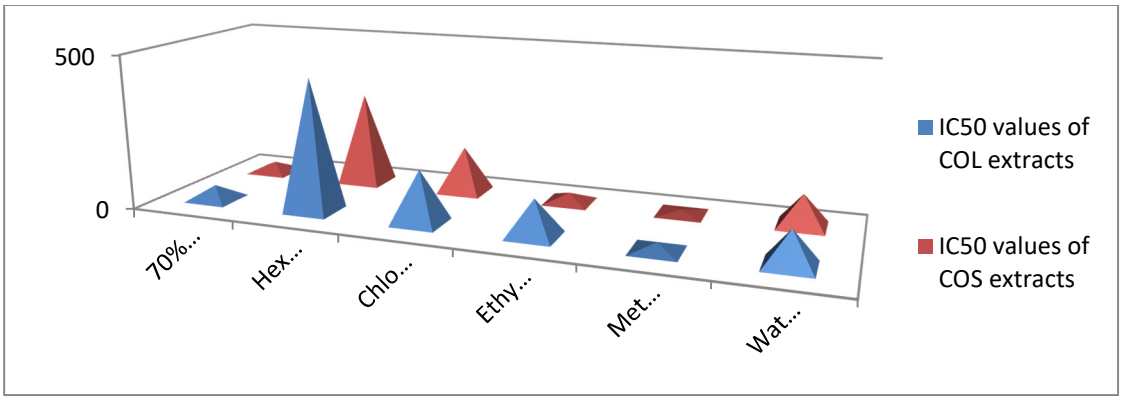

Figure-5: Results of Comparison of IC_{50} Values of Different extracts of *C. ovalifolium* leaf and stem

CONCLUSION:

The research work was done to evaluate the efficacy of the medicinal properties and nutritional values of *C. ovalifolium*. From the all the analysis we would like to conclude that methanol and 70% solvents are a better choice for extraction of this plant as well as methanol and 70% ethanol extracts possess radical scavenging potential among the all extracts. The presence of flavonoid and phenolic compounds in high percentage is a one of the main reason for the radical scavenging property and medicinal value of the plant extracts.. Present work gives the idea of the basic preliminary research in phytochemistry field and the future work is aimed to isolate the flavonoids and phenolic compounds from a methanolic extract of the plant *Combretum ovalifolium* which are responsible for the antioxidant activity.

Conflicts of interest:

The authors have declared no conflicts of interest.

REFERENCES:

1. Raji Muthuraja, Veeraiyan Nandagopalan, Binu Thomas, Chitrambalam Marimuthu. An Ethno-Botanical survey of medicinal plants used by kolli Malayalis of Namakkal district Eastern Ghats Tamil Nadu India. Eur J Environ Ecology 2014; 1(1): 33-43.
2. Mafatlal M Kher, Nataraj M. Micropropagation of *Combretum ovalifolium Roxb*.: a medicinally important plant. Rendiconti Lincei-Scienze Fisiche E Naturali 2017; DOI 10.1007/s12210-017-0625-z.
3. Rajendra Kumar Jain. Fuelwood Characteristics of Medium Tree and Shrub Species of India. Bioresour Technol 1994; 47: 81-4.
4. Retrieved from: https://eol.org/pages/5427253 accessed 12th December 2018.
5. Basalingappa L Hungund, Chandravadan H Pathak. A survey of plants in Gujarat India for alkaloids saponins and tannins. U.S.D.A. Forest Service Research Paper NE-201 1971;201.

https://www.fs.fed.us/ne/newtown_square/publications/research_papers/pdfs/scanned/OCR/ne_rp201.pdf accessed 27th March 2019.

6. Kamala Pranoothi E, Narendra K, Suman Joshi D.S.D, Swathi J, Sowjanya K M, Rathnakarreddi KVN, et al.,. Studies on qualitative, quantitative, phytochemical analysis and screening of *in vitro* biological activities of *Leucas indica* (L) *VAR* Nagalapuramiana. Int J Herb Med 2014; 2 (3): 30-6.
7. Raaman N. Phytochemical techniques. 1st ed. New India publication Agency New Delhi: Jai Bharat Printing press; 2006. p.19 – 24.
8. Edeoga1 H O, Okwu D E, Mbaebie B O. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol 2005; 4 (7): 685-8.
9. Sathya V, Bharathidasan R, Tamil Selvi S, Sophia Rebeccal N, Ilakkiya R, Prabakaran M. Quantitative qualitative phytochemical analysis and *in vitro* antibacterial activity of *Bauhinia tomentosa* L. J Nat Prod Plant Resour 2013; 3(2):31-6.
10. Landan Z, Amupitan J O, Oyewale O A, Ayo R G, Temple E, Ladan E O. Phytochemical screening of the leaf extracts of *Hyptis spicigera* plant. Afr J Pure Appl Chem 2014; 8(5): 83-8.
11. Harborne JB. A Guide to modern techniques of plant Analysis.2nd ed. USA: Kluwer Academic Publisher; 1998; 18-9.
12. Nazish Siddiqui, Abdur Rauf, Abdul Latif, Zeenat Mahmood. Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal Unani drug Gul-e-Zoofa (Nepeta bracteata Benth). J Taibah Univ Med Sci 2017; 12(4): 360-3.
13. John de Britto A, Herin Sheeba gracelin D, Benjamin Jeya Rathna Kumar P. Qualitative and quantitative analysis of phytochemicals in *marsilea minuta* linn. Int J Pharma Bio Sci 2013; 4(1): 800 – 5.
14. Malik S K, Ahmad M., Khan F. Qualitative and quantitative estimation of terpenoid contents in some important plants of Punjab Pakistan. Pak J Sci 2017; 69(2):150-4.
15. Geetha T S, Geetha N, Phytochemical Screening, Quantitative Analysis of Primary and Secondary Metabolites of *Cymbopogan citratus (DC) stapf*. leaves from Kodaikanal hills Tamilnadu. Int J Pharmtech Res 2014; 6(2): 521-9.
16. Retrieved from: http://shodhganga.inflibnet.ac.in/bitstream/10603/143633/13/13_chapter%204.pdf accessed 12th December 2018; 140-74.
17. Abiodun falodun, Emmanuel E I Irabor. Phytochemical proximate antioxidant and free radical scavenging evaluations of *calliandria surinamensis*. Acta Pol Pharm Drug Res 2008; 65(5): 571-5.
18. Archa Vermani, Navneet, Prabhat, Avnish Chauhan. Physico-Chemical Analysis of Ash of Some Medicinal Plants Growing in Uttarakhand India. Nat Sci 2010; 8(6):88-91.
19. Adinortey M B, Sarfo J K, Quayson E T, Weremfo A, Adinortey C A, Ekloh W, Ocran. Phytochemical screening proximate and mineral composition of *Launaea taraxacifolia* leaves. Res J Med plant 2012; 6(2): 171-9.
20. Badakhshan Mahdi-Pour, Subramanion L Jothy, Lachimanan Yoga Latha, Yeng Chen, Sreenivasan Sasidharan. Antioxidant activity of methanol extracts of different parts of Lantana camara. Asian Pac J Trop Biomed 2012; 2(12): 960-5.

21. Rajurkar NS, Hande SM. Estimation of phytochemical content and antioxidant activity of some selected traditional Indian medicinal plants. Indian J Pharm Sci. 2011; 73(2):146–51.
22. Ranjan, S., R. Matcha, G. K. Saride. In vitro antiinflammatory and antioxidant activity of leaf extracts of *Datura Metal*. Asian J Pharm Clin Res. 2013; 6 (8): 146-9.
23. Irda Fidrianny, Agung Darmawati, Sukrasno. Antioxidant capacities from different polarities extracts of cucurbitaceae leaves using FRAP, DPPH assays and correlation with phenolic, flavonoid, carotenoid content. Int J pharm pharm Sci 2014; 6 (2): 858-62.