ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 4, pp: (279-290), Month: October - December 2018, Available at: www.researchpublish.com

Evaluation of phytochemical and antioxidant analysis of *Memecylon flavescens* - an endangered taxon

[1]*D.H.Tejavathi, [1]B.S.Sumalatha, [1]R. Nijagunaiah, [2]P.Anitha, [3]K.Gayatramma

[1]Department of Botany, Bangalore University, Bangalore-560 056, Karnataka, India
[2]Department of Botany, BMS College for Women, Basavangudi, Bangalore-560 025, Karnataka, India
[3]Department of Botany, MES College, Malleshwaram, Bangalore-560 003, Karnataka, India
*Corresponding author: E-mail: tejavathi_hanu@yahoo.com

Abstract: **Medicinal plants play a major role in the discovery of new therapeutic agents for drug development. In recent times, bioactive compounds derived from plants have a dynamic role to play in prevention of various human diseases. *Memecylon flavescens*, is an endangered taxon belongs to the family Melastomataceae. The present study is an attempt to investigate the presence of phytochemicals in methanolic extracts of stem and leaf by GC/MS and to estimate the content of total phenols and flavonoids. In addition, antioxidant potential of the extracts was analysed by various standard *in vitro* assays. Results have shown that stem contains 32.73 ± 0.079 mg/g of phenols and 13.56 ± 0.062 mg/g of flavonoids, while the extract of leaf has 25.89 ± 4.97 mg/g of phenols and 10.18 ± 0.318 mg/g of flavonoids. The GC/MS analysis has revealed the presence of 37 compounds in stem and 27 compounds in leaf. A few compounds are found to be common in both the extracts with 2(5H)-Furanone, 3-Methyl-5-Methylene, a ketone group as a major compound with peak area of 19.20 % in stem extract and 33.82% in leaf extract. Whereas, 3, 5-Dimethoxy-4-Hydroyphenethylamine, a amine group has shown a least percent of peak area of 0.45% in stem extract and 4-Methyl-2, 5-Dimethoxybenzaldehyde, an aldehyde group with 0.56% in leaf extract. The extracts were screened for potential antioxidant activity by DPPH, ABTS, and reducing power capacity. Free radical scavenging potentials of the extracts at various concentrations were tested. The stem extract has shown higher scavenging activity than the leaf extract. A strong reducing power capacity was observed for methanolic extract of stem. The antioxidant potential of the stem and leaf extracts were correlated with the total phenols and flavonoids. The study has highlighted the antioxidant potential of the stem extract, thereby exploring the promising application in pharmaceuticals.**

***Keywords: Memecylon flavescens*, Endangered, GC-MS, Phytochemical, Antioxidant.**

1. INTRODUCTION

The rich floristic diversity in India is representing 11.4% of the total recorded plant species of the world flora and about 28% of endemic species [1]. Nearly 77 plant species are critically endangered according to IUCN Red list of threatened species, version 2016-3 [2, 3]. The number of threatened species is steadily increasing in recent years mainly because of their habitat loss. Recovery of the endangered species is need of the hour by restoring their ecosystem. In addition to their conservation, exploring the medicinal potentialities is also urgently needed since the state of many known medicinal plants is precarious because of unscientific collection [3].

Memecylon flavescens belongs to the family Melastomataceae, is endemic to Nilgiri biosphere and categorised as Endangered with an IUCN ID 31203. The genus *Memecylon* is represented by around 250 species in the world [4]. Among them, about 49 species, of which 23 are endemic, are reported from India [5, 6]. Gamble described this species for the first time, based on his collection from Sispara in 1884 (2133 m), Avalanche in 1885 (2286 m) and Kundah in 1885 (2133 m) [7]. All these localities are presently included in Nilgiri Biosphere Reserve, Tamilnadu.

The species of *Memecylon* have shown potential pharmacological activities such as anti-inflammatory, antidiabetic, antiviral, hepatoprotective, antimicrobial and antioxidant activity where exact mechanism of action by bioactive compounds is not known, but being treated in curing the skin aliments and herpes [8]. Isolation and characterization of these bioactive chemicals is prerequisite for further biological and pharmacological studies. One of the important phytochemicals is polyphenols, which are thought to be largely responsible for the medicinal properties and health benefits of medicinal plants [9]. The polyphenols are mainly synthesized by plants to face the various challenges of the environment in addition to their role in plant development. Most of the polyphenolic compounds are known for their antioxidant potential that neutralize the effects of antioxidative stress. *M. flavescens* is underexploited except a few reports in taxonomic literature regarding its description and a solitary report on phytochemical studies [8]. However, a few other species of *Memecylon* have attracted researchers because of their medicinal properties [8, 10-15]. With this background, GC-MS and antioxidant studies have been carried out to identify the different bio components present in the methanolic extracts of leaf and stem of Avalanche populations, and to study the antioxidant potential by employing various antioxidant assays.

II. MATERIALS AND METHODS

Identification, collection, and preparation of *Memecylon flavescens* extract:

The leaves and stem material were collected from Avalanche, Reserve forest of Niligiri district, Tamil Nadu and BSI, Coimbatore, authenticated the identification. The voucher specimen is deposited in Department of Botany, Bangalore University (**Fig 1a-d)**. The collected samples were cleaned and dried under shade at room temperature and powdered with the help of laboratory grinder. Extraction was carried out using 10g of each sample in Soxhlet apparatus for 12 h. The solvent used was methanol. The methanol extract was evaporated to dryness. The dried powder was stored at 4° c for further use in GC-MS analysis, quantification of polyphenols and for antioxidant analysis. For preliminary screening of phytochemicals, one gram of the powdered samples was soaked in different solvents in a stopper container at room temperature for 24 h. The mixture was then filtered to remove the debris and the filtrate was used for screening various phytochemicals.

Preliminary qualitative analysis of leaf and stem extracts of *M. flavescens:*

Preliminary screening for the presence and absence of various phytochemicals were conducted by following the standard methods [16, 17].

Test for Alkaloids: Extracts were dissolved individualy in dil. HCl and filtered.

Mayer's test: Filtrates were treated with Mayers reagent (Potassium Mercuric iodide), formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's test: Filtrates were treated with Wagner's reagent (iodine in potassium iodide), formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's test: Filtrates were treated with Dragendroff's reagent (solution of potassium bismuth iodide), formation of red precipitate indicates the presence.

Test for Carbohydrates: Extracts were dissolved individually in 5 ml distilled water, filtered, and tested further.

Molisch's test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in test tube. Formation of the violet ring at the junction indicates the presence.

Benedict's test: Filtrates were treated with Benedicts reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehlings test: Filtrates were hydrolysed with dil. HCl, neutralised with alkali and heated with Fehlings A & B solution. Formation of red precipitate indicates the presence of reducing sugar.

Test for Saponins:

Froth test: Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 min, formation of 1 cm layer of foam indicates the presence of saponins.

Foam test: 0.5 gm of extracts was shaken with 2 ml of water. If foam persists for 10 min, it indicates the presence.

Test for phytosterols:

Salkowski's test: Extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of con. sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Liberman Burcharsds test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled, and cooled. Con sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Test for phenols:

Ferric chloride test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicated the presence of phenols.

Test for tannins:

Gelatin test: 1% gelatine solution containing 10% sodium chloride was added to the extracts. Formation of white precipitate indicates the presence of tannins.

Test for Flavonoids:

Alkaline reagent test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour becomes colourless on addition of dil.Hcl , indicates the presence of flavonoids.

Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Test for proteins and amino acids:

Xanthoproteic test: The extracts were treated with few drops of con nitric acid; formation of yellow colour indicates the presence of amino acids.

Ninhydrin test: 0.25% w/v ninhydrin reagent was added to the extracts and boiled for few minutes, formation of blue colour indicates the presence of amino acids.

Test for coumarin:

3 ml of 10% NaOH was added to 2 ml of aqueous extract, formation of yellow colour indicates the presence of coumarin

GC-MS analysis:

Methanolic extracts of stem and leaf of *M.flavescens* were analysed by the GC-MS technique. Chemical composition of the crude methanolic extracts was determined using Shimadzu QP 2010S with a Rxi-5Sil MS column (30 m length x 0.25 mm ID x0.25 µm thickness). Samples were injected under the following conditions: Helium was used as carrier gas at approximately 1 ml/min at pulsed splitless mode. The sampling time was 2 min and one µl was the injection volume. The injection temperature was maintained at 260^{o}C.The column flow was 1.00mL/min, whereas the total flow was 104.0mL/min. The oven temperature was programmed as follows: started at 60°C then elevated to 280°C at a rate of 5°C/min, with a 2min hold at 280°C. The ion source and the interface temperatures were set at 200° C and 280° C respectively. The mass spectrophotometric detector was operated in electron impact ionization mode with an ionizing energy of 70 eV and scanning from m/z 50-500 at the scan speed of 1000. The event time for mass spectra was 0.50. Spectral data from Wiley 8 and NIST 11 libraries were used in the identification of the separated peaks obtained for the samples.

Total Phenolic contents:

The total phenolic content in the methanolic extracts of leaf and stem were determined using Folin–Ciocalteu method [18] with slight modifications. 0.2 ml of 2N Folin –Ciocalteu reagent was added to leaf, stem extracts (1mg/ml), and 2 ml of sodium carbonate (7.0% w/v) was added after keeping for 5 min at room temperature. Then, the mixture was made up to 10 ml with distilled water and allowed to stand for 90 min. The mixture was kept in dark with intermittent shaking. The absorbance was measured at 765 nm using UV-Vis spectrophotometer (Elico, SL 164). The experiment was carried out in triplicate. Gallic acid was used as standard (10-100 µg/ml, r^2=0.999) and total phenolic compounds concentration in leaf and stem extracts are expressed as milligram of Gallic acid equivalent per g (mg GAE/g).

Total flavonoids contents:

Total flavonoids content was determined by Aluminium chloride method [19]. 3 ml of methanolic extracts of leaf and stem were mixed with 0.2 ml of aluminium chloride (10%), and 0.2 ml potassium acetate (1M) and the final volume was made up to 9ml by addition of 5.6 ml of distilled water. After incubating at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm using UV-Vis spectrophotometer (Elico, SL 164). The blank was prepared by replacing methanol extract with distilled water (10-100 µg/ml, r^2=0.990). The final absorbance of each sample was compared with a standard curve plotted against Quercetin. The total content of flavonoids content was expressed in milligrams of Quercetin equivalent (mg QEE/g).

Antioxidant assays:

(i) *Total antioxidant assay-Phosphomolybdate assay*

The total antioxidant activity of the extract was estimated by phosphomolydeum method as described by Prieto et al. [20]. The principle that is involved in this assay is the reduction of Mo (VI)–Mo (V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. Tubes containing 0.3 ml of extract with 3 ml of reagent solution that was prepared from 0.6 M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate were incubated at 95°C for 90 min. Then the absorbance was measured at 695 nm against blank after cooling at room temperature. Methanol (0.3 ml) in the place of extract is used as the blank and the activity is expressed as the number of equivalents of ascorbic acid in mg per g of the extract.

(ii) *DPPH radical- scavenging activity*

Free radical scavenging activity of leaf and stem extracts was determined by Braca *et al* [21] method with modifications. 0.1 ml of methanolic extracts and control at various concentrations ranging from 25 to 100µg/ml were added to 2 ml of 0.002% methanolic solution of DPPH. After 30 min of incubation in dark, absorbance was recorded at 517 nm. Ascorbic acid was used as standard. The percentage of scavenging activity was calculated by using the equation

$$\%\ \text{scavenging activity} = \frac{\text{Ac} - \text{As}}{\text{Ac}} \text{x} 100$$

Where Ac – absorbance of control and As- absorbance of samples. The activity was reported as IC_{50} that represent the concentration of the extract needed to scavenge 50% of DPPH free radicals.

(iii) *ABTS radical cation scavenging method.*

The assay was determined with minor modifications of Re et al. [22] method. ABTS radicle cation is produced when ABTS aqueous solution (2mM) reacts with 17 mM potassium persulfate. The mixture was allowed to stand in dark at room temperature for 12-16 h before use. The ABTS*+ solution was diluted with methanol until an absorbance of 0.7±0.01 is reached at 734nm. 2 ml of this diluted ABTS reagent was added to 0.1ml of extracts. Ascorbic acid was used as standard. The absorbance was read at 734nm and percent inhibition was calculated.

$$\%\ \text{scavenging activity} = \frac{\text{Ac} - \text{As}}{\text{Ac}} \text{x} 100$$

The IC_{50} values of the extracts were determined, plotting the percentage of scavenging activity versus the concentration of extract using linear regression analysis. Lower the IC_{50} value, higher the radical scavenging effect.

(iii) *Reducing power capacity*

The assay was conducted according to the method of Oyaizu [23]. The different quantities of methanolic extracts of leaf and stem (20- 100 µg) in 1 ml of methanol were mixed with 2.5 ml of phosphate buffer at the concentration of 0.2 M at pH 6.6. To this mixture, 2.5ml of potassium ferricyanide (1%) was added. The mixture was incubated at 50°c for 20 min and then cooled. 2.5ml of 10% trichloroacetic acid (2.5 ml) was added to the mixture and centrifuged at 3000 rpm for 10 min. 2.5ml of upper layer of the solution was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (0.1%). The absorbance was measured at 700 nm using UV-Vis spectrophotometer (Elico, SL 164). Increased absorbance of the reaction mixture indicated increased reducing power.

III. RESULTS AND DISCUSSION

Phytochemicals are the chemical compounds synthesized by plant either through primary or secondary metabolism. They play a vital role in plant growth and defence mechanism. Majority of these bio chemicals are reported to have anticancerous, antimutagenic, anti-inflammatory and antioxidant properties. Isolation and identification of these bioactive chemicals is the need of the hour that lead to further biological and pharmacological studies. Polyphenols comprises a large number of bioactive compounds and known for their antioxidant potential. The present study is an attempt to analyse the methanolic extracts of stem and leaf of *Memecylon flavescens* by GC-MS for the presence of active phytochemicals and to detect antioxidant potential by employing various assays. The extract percentage of yield was varied greatly among the different solvents used with the highest one was recorded for methanol. Highest percent of yield was obtained in the methanolic extract of stem compared to leaf; 5.14 g in stem and 4.2 g in leaf samples. The yield from the other solvents was less than one gram. Comparison of extraction yield in different extraction solvents revealed that solvent type had a significant effect on the extraction yield. Methanol is considered as better solvent for more consistent extraction of active compounds from medicinal plants than other solvents [24-26]. Aqueous, methanol, ethyl acetate, hexane and butanol extracts of leaf and stem were subjected to different tests. Preliminary screening of the extracts revealed the presence of various phytochemicals as shown in **Table1.** Among the solvents, methanol was found to be more effective in detecting alkaloids, flavonoids, tannins, phenols, terpenoids and coumarins than other solvents. Saponins were not detected in both leaf and stem extracts of all the solvents. However saponins were identified in leaf extracts of *Memecylon edule*, *M.malabaricum* and *M.talbotianum* [10, 13]

Since methanol extract has produced maximum yield, it was analysed by GC-MS. A total of 37 in stem and 27 different compounds in leaf extract were identified. The identified compounds with their molecular formula, compound group, retention time and peak area percent are presented in **Table 2&3**. However, GC-MS profile of leaf extract of *Memecylon umbellatum* and *M. edule* have revealed 34 and 26 major compounds of medicinally valuable respectively[11, 14]. While Diris *et.al.*, [15] have reported similar chemical compounds in two *Melastoma* species from GC-MS analysis and concluded close relatedness of both the species based on the phytochemical studies. Among the identified compounds in the present study, carboxylic groups are predominant in both the extracts. 13 carboxylic group compounds in stem extract and 8 in leaf extract were identified. Highest percent of peak area was shown for 2(5H)-Furanone, 3-Methyl-5-methylene in both stem (19.20%) and leaf extracts (33.82%). Whereas, 3, 5-Dimethoxy-4-Hydroxyphenethylamine in stem and 4-methyl-2, 5-Dimethoxybenaldehyde in leaf extract were found to be least with 0.45% and 0.56% respectively. The chromatogram of leaf and stem extracts with the retention time and the detected peaks correspond to the phytochemicals present in the extracts. NIST 11 and Wiley 8 libraries were used to identify the compounds based on peak and retention time (**Fig.2&3).** Most of the compounds having carboxylic group are said to have antioxidant potential [27].

Chemical structure of the polyphenols is ideal for scavenging free radicals and for creating chelates with metal ions, which makes them effective antioxidants [28]. Keeping in view the fact that polyphenols containing carboxylic group in their structure possess an excellent antioxidant activity, the extracts of stem and leaf are subjected to the estimation of total phenols and flavonoids. Further, the antioxidant potential of these extracts were analysed by various assays. Polyphenols have been reported to be responsible for the antioxidant potential of botanical extracts [29].

The amount of total phenols and flavonoids as estimated by Folin–Ciocalteu method and Aluminium chloride colorimetric method respectively are found to be more in stem extract than leaf. The content of total polyphenols in the stem and leaf methanolic extracts estimated by Folin-ciocalteu methods is expressed as milligram of gallic acid equivalent (GAE). The stem extract has more content of total polyphenols than the leaf extract. 32.73 ± 0.079 GAE mg/g of total polyphenols was recorded in stem extract, whereas 25.89 ± 4.97 GAE mg/g in leaf extract.

The flavonoid content was estimated by Aluminium chloride colorimetric method and it is expressed as milligram of Quercetin equivalent per gram. The total flavonoid content of methanolic extract of stem was found to be more with 13.56 ± 0.062 than the leaf extract, which contain 10.18 ± 0.318.This observation is correlated with GC-MS results where stem extract has more number of bio active compounds containing carboxyl groups than the leaf. The total antioxidant assay correlates the data obtained by GC-MS revealing significantly more antioxidant activity of stem extract than the leaf extract. The phosphomolybdenum method was used to evaluate the total antioxidant activity of extracts and is expressed as ascorbic acid equivalent. Stem extract has exhibited higher level of antioxidant potential of 15.37 ± 0.02 mg AAE/g than leaf extract which was showing 13.06 ± 0.031 mg AAE/g.

Total phenol and flavonoid contents in the Sispara population of *M. flavescens* were found to be less than Avalanche population of present study [12]. Correspondingly, DPPH radical scavenging activity of the extracts was determined based on the ability of extract to reduce the stable radical thereby changing the colour of the DPPH solution from purple to yellow. Stem and leaf extracts were evaluated at different concentrations ranging from 25 µg/ml to 100 µg/ml. Percent of Radical scavenging activity of the extracts were found increasing as the concentration increases. Among the two samples, methanolic extract of the stem exhibits maximum free radical scavenging activity ranging from 62.54± 0.04 % to 85.56± 0.06 % with IC_{50} value of 48.22 µg/ml. Whereas the leaf extract has shown the free radical scavenging activity ranging from 58.73 ± 0.02 % to 74.13 ±0.009 with IC_{50} value of 60.47 µg/ml **(Fig.4).** The IC_{50} of Sispara population is reported as 115.17 ± 0.04 [12], whereas in the present study, the IC_{50} values of stem and leaf extracts are found to be 48.22 and 60.47 respectively in DPPH assay to analyse the antioxidant potential. ABTS assay measures the relative ability of the extracts to scavenge the ABTS free radicals as compared to ascorbic acid is used which used as a standard. Colour changes from blue colour to colourless indicates the scavenging capacity of the extracts. Percent of scavenging activity ranges from 61.80 ± 0.001 to 79.93 ± 0.002 in stem extracts and 48.11 ± 0.004 to 62.43 ± 0.004 in leaf extracts with IC_{50} value of 47.89µg/ml and 59.11µg/ml respectively **(Fig.5).** In both the assays, stem extract has better scavenging activity than leaf extract. However, Kopjar et al [30] have observed different values for the various methods employed to detect the antioxidant potential of selected taxa. They are of the opinion that different phenolic compounds react differently depending on the applied reagent for antioxidant determination [29, 30].

The reducing power capacity assay depends on the reducing capacity of the extract from ferric($Fe3^{+)}$ to ferrous ($Fe2^{+}$), thereby changing the colour of the reaction mixture from yellow colour to pale green or blue colour depending on the concentration. Ascorbic acid was used as standard. The reducing power of the extracts increased with increase in concentration. Higher absorbance of the reaction mixture indicates higher reduction potential. Stem extract has shown better reducing capacity than the leaf extract in the present study. The reducing capacity of the compound may serve as a significant indicator of its potential antioxidant activity.

Presence of polyphenols, which includes flavonoids, is responsible for the antioxidant potential of the biological materials. Regardless of the method employed, quantification of phenols and flavonoids correlates with the results obtained by DPPH, ABTS and reducing power assays carried out to find out the antioxidant potential of the stem and leaf extracts of Avalanche populations of *M. flavescens*.

IV. CONCLUSION

Memecylon flavescens is an endangered and underexploited taxon. Since other species of *Memecylon* were reported to have medicinally important compounds and antioxidant potential, the present study is an attempt to find out its medicinal importance through GC-MS analysis and various antioxidant assays using the methanolic extract of stem and leaf. Preliminary phytochemical and GC-MS analysis have revealed the presence of medicinally important compounds in both the extracts. Antioxidant assays have confirmed that both the extracts have significant potential to scavenge the free radicals. Present findings can be further exploited by employing precise pharmacological studies.

ACKNOWLEDGEMENT

We are thankful to the Ministry of Forests, Environment, and Climate Change (MoEFCC), New Delhi for funding this project and all the Forest officials who helped us in the survey to identify the species and locating them.

REFERENCES

[1] Singh P, Dash SS, "Plant discoveries 2013- new genera, species, and new records". Botanical Survey of India, Kolkata. 2014.

[2] IUCN, "T he IUCN Red list of Threathened Species" Version 201-3; http://www.iucnredlist.org

[3] Ravikanth G, Jagadish MR, Vasudeva R, Uma Shaanker R, Aravind NA (2018) Recovery of Critically endangered plant species in India: need for a comprehensive approach. Curr Sci, 114:504-511.

[4] Mabberley DJ, "Mabberley's Plant-Book: A Portable Dictionary of Plants, their Classification and Uses": 3rd ed., Edition. Cambridge University Press : UK, 2008.

[5] Das Das M, Maity D, Pramanik A (2015) Typificaiton of the Name *Memecylon talbotianum* Brandis (Melastomataceae). Phytotaxa.203:210-212.

[6] Murugan C, Gopalan R (2006) Four additions to Indian *Memecylon* L. (Melastomataceae) from South India. Indian J Forest, 29:105-108.

[7] Gamble, J.S."Flora of the Presidency of Madras". Vol. 1.Third edition. Adlard and Son,Ltd.London, 1957. pp.503.

[8] Bharathi TR, Sampath Kumara KK, Prakash HS (2016) *Memecylon* species: A review of traditional information and taxonomic description. International Journal of Pharmacy and Pharmaceutical Sciences. 8:26-34.

[9] Tabassum S, Khare S, Jain K (2016) Spectrophotometric Quantification of Total Phenolic, Flavonoid and Alkaloid content of *Abrus precatorius* L. Asian J. Pharmaceutical and Clinical Research, 9: 371-374.

[10] Elavazhagan T, Arunachalam KD (2010) Phytochemical and Antibacterial Studies of Seed Extracts of *Memecylon edule*. International Journal of Engineering Science and Technology. 2:498-503.

[11] Murugesan S, Panneerselvam A (2013) Evaluation of phytochemical constituents from stems of *Memecylon umbellatum* Brum. F. by GC-MS analysis. Research and Reviews: Journal of Botanical Sciences, 2: 29-34.

[12] Sivu AR, Pradeep NS, Ramesh Kumar KB, Pandurangan AG (2013) Evaluation of phytochemical, antioxidant and antimicrobial activities of *Memecylon* L. species from Western Ghats. Indian Journal of Natural Products and Resources, 4: 363-370.

[13] Vivek MN, Kambar Y, Prashith Kekuda TR, Manasa M, Raghavendra HL (2014) Antimicrobial and Radical Scavenging Activity of *Memecylon malabaricum* (C.B.Clarke) Cogn and *Memecylon talbotianum* Brandis. Sci. Technol.Arts Res.J, 3:174-179.

[14] Srinivasan R, Natarajan D, Shivakumar MS. (2014) Antimicrobial and GC-MS Analysis of *Memecylon edule* leaf extracts. International J. Current Pharmaceutical Review and Research, 5:1-13.

[15] Diris MN, Basri AM, Metali F, Ahmad N, Taha H (2017) Phytochemicals and Antimicrobial Activities of *Melastoma malabathricum* and *Melastoma beccarianum* Leaf Crude Extracts. Res J Phytoche, 11: 35-41.

[16] Trease GE, and Evans WC, "Pharmacognosy",11th (ed.). ELBS/Bailliere Tindall, *London*. 1989, pp. 45-50.

[17] Sofowora EA, "Medicinal plants and Traditional medicine in Africa" Spectrum Books Ltd, Ibadan, Nigeria. 1993, pp. 191-289.

[18] Singleton VL, Rossi JA Jr. (1965) Colorimetric of total phenolics with phosphomolybdic – phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16: 144-158.

[19] Chang C, Yang M, Wen H, Chern J (2002) Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. Journal of Food and Drug Analysis, 10: 178-182.

[20] Prieto P, Pineda M, Aguilar M (1999). Spectrophotometric quantification of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E^1. Analytical Biochemistry. 269: 337-341.

[21] Braca A, Tommasi ND, Bari LD, Pizza C, Politin M, Morelli I (2001) Antioxidant principles from *Bauhinia tetrapotensis*. Journal of Natural Products, 64: 892-895.

[22] RE R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26: 1231-1237.

[23] Oyaizu M (1986). Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition, 44: 307-315.

[24] Shahidi F, Janitha PK, Wanasundara PD (1992) Phenolic antioxidants. Critical Reviews of Food Science and Nutrition, 32:67-103.

[25] Eloff JN (1998) Which extractant should be used for the screening and isolation of antimicrobial components from plants. Journal of Ethnopharmacology. 60:1-8.

[26] Elangovan K, Priyanka D, Anupriya S, Banu ZS, Murugesan K (2014) Evaluation of *In vitro* antioxidant and GCMS spectroscopic analysis of *Memecylon Umbellatum* Brum F. for its bioactive compounds. International Journal of Pharmaceutical Development and Technology, 4:225-234.

[27] Velika B, Kron I (2012) Antioxidant properties of benzoic acid derivatives against superoxide radical. Free Radicals and Antioxidants, 2: 62-67.

[28] Rzepecka-Stojko A, Stojko J, Kuvek-Gorecka A, Gorecki M, Kabala-Dzik A, Kubina R, Mozdzierz A, Buszman E (2015) Polyphenols from Bee Pollen: Structure, Absorption, Metabolism and Biological Activity. Molecules, 20: 21732-21749.

[29] Wong SP, Leong LP, Koh JHW (2006) Antioxidant Activities of Aqueous Extracts of Selected Plants. Food Chemistry, 99: 775-783.

[30] Kopjar M, Tiban NN, Pilizota V, Babic J (2009) Stability of anthocyanins, phenols, and free radical scavenging activity through sugar addition during frozen storage of blackberries. Journal of Food Processing and Preservation, 33: 1-11.

APPENDIX-A

Fig.1. *Memecylon flavescens* a. Plant-habit b. Twig with Inflorescence c. Flower d. Mature Fruit

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 4, pp: (279-290), Month: October - December 2018, Available at: www.researchpublish.com

Table 1: Preliminary screening of stem and leaf extracts of *M.flavesences* for various phytochemicals

Chemical Test	Methanol		Ethyl Acetate		N-Hexane		Butanol		Aqueous	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Alkaloids	+	+	-	+	+	+	-	-	+	-
Flavanoids	-	+	+	-	+	-	+	-	-	-
Tannins	-	+	+	-	+	-	+	-	-	-
Phenols	+	+	+	-	+	-	+	-	-	+
Terpenoids	-	+	-	+	+	+	+	+	-	-
Saponins	-	-	-	-	-	-	-	-	-	-
Coumarins	-	+	-	+	-	+	-	-	+	+
Carbohydrates	+	+	-	-	-	-	-	-	+	+
Phytosterols	-	+	-	+	+	+	+	-	+	+
Protiens and Amino Acids	+	+	+	+	+	+	-	-	+	-

Table 2: List of chemical compounds characterised in the stem sample of *M. flavescens* through GC-MS analysis

Sl. no	Stem sample	Molecular Weight g/mol	Molecular formula	Compound group	Rt	Peak area %
1	Butane,1-(ethenyloxy)-3-methyl-	114.1855	$C_7H_{14}O$	Ether and alkene	6.908	0.90
2	2(5H)-Furanone, 3-Methyl-5-Methylene	110.11100	$C_6H_6O_2$	Ketone	7.397	19.20
3	N-(3-Butenyl)-N-Methylcyclohexanamine	167.296	$C_{11}H_{21}N$	Amine	9.080	0.78
4	2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4h-Pyran-4-One	144.1253	$C_6H_8O_4$	Ketone	10.763	0.73
5	5-Hydroxymethylfurfural	126.111	$C_6H_6O_3$	Aldehyde	13.277	8.25
6	2,3-2H-4-Methyl-imidazole-2-one	98.105	$C_4H_6N_{20}$	Imidazole (NH)	15.166	5.51
7	3-Ethyl-3-Heptanol	144.258	$C_9H_{20}O$	Alcohol	15.700	1.08
8	4-Hydroxy-3,5,5-Trimethyl-2-Cyclohexen-1-One	154.209	$C_9H_{14}O_2$	Ketone	16.192	1.89
9	Dodecanoic Acid	200.3178	$C_{12}H_{24}O_2$	Carboxylic	21.707	3.23
10	Beta-D-Glucopyranose,1,6-Anhydro-	162.141	$C_6H_{10}O_5$	Aldehyde	22.133	7.03
11	Cyclopropanecarboxylic Acid,2,2-Dimethyl-3-(2-Methyl-1-Propenyl)-	302.4079	$C_{19}H_{26}O_3$	Carboxylic	22.925	0.63
12	Benzeneacetic Acid, 4-Hydroxy-3-Methoxy-	182.1733	$C_9H_{10}O_4$	Carboxylic	23.601	1.03
13	2,4,6(1H,3H,5H)-Pyrimidinetrione,5-Acetyl	170.124	$C_6H_6N_2O_4$	Ketone	23.975	2.19
14	1,6-Anhydro-Beta-D-Glucofuranose	162.141	$C_6H_{10}O_5$	Alcohol	24.478	2.68
15	Heptacosanoic Acid, Methyl Ester	284.484	$C_{18}H_{36}O_2$	Carboxylic , ester	25.300	1.63
16	Tetradecanoic Acid	228.3709	$C_{14}H_{28}O_2$	Carboxylic	26.169	1.05
17	Ethanone,1-(4-Hydroxy-3,5-Dimethoxyphenyl)-	196.1999	$C_{10}H_{12}O_4$	Ketone	27.310	0.66
18	Neophytadiene	278.524	$C_{20}H_{38}$	Alkene	27.700	1.53
19	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	296.539	$C_{20}H_{40}O$	Alcohol	28.575	0.60
20	3,5-Dimethoxy-4-Hydroxyphenethylamine	233.692	$C_{10}H_{16}ClNO_3$	Amine	28.766	0.45

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 4, pp: (279-290), Month: October - December 2018, Available at: www.researchpublish.com

21	Hexadecanoic Acid, Methyl Ester	270.4507	$C_{17}H_{34}O_2$	Carboxylic , ester	29.497	0.63
22	9,12-Octadecadienoic Acid, Methyl Ester, (E,E)-	294.4721	$C_{19}H_{34}O_2$	Carboxylic , ester	29.692	2.00
23	Hexadecanoic Acid	256.43	$C_{16}H_{32}O_2$	Carboxylic	30.370	6.06
24	Cis-13-Octadecenoic Acid, Methyl Ester	296.495	$C_{19}H_{36}O_2$	Carboxylic, ester	32.824	1.18
25	Cis-Vaccenic Acid	282.468	$C_{18}H_{34}O_2$	Carboxylic	33.667	8.00
26	Octadecanoic Acid	284.484	$CH_3(CH_2)_{16}COOH$ or $C_{18}H_{36}O_2$	Carboxylic	34.052	0.95
27	Trichloroacetic Acid, Hexadecyl Ester	387.81	$C_{18}H_{33}Cl_3O_2$	Carboxylic	39.575	0.70
28	Hexadecanoic Acid,2-Hydroxyl-1-(Hydroxymethyl)Ethyl Ester	330.509	$C_{19}H_{38}O_4$	Carboxylic	39.800	0.96
29	Pentacosane	352.691	$C_{25}H_{52}$	Alkane	42.886	1.25
30	Stigmast-5-EN-3-0l.(3.Beta)-	410.686	$C_{29}H_{46}O$	Ketone	44.798	8.58
31	Squalene	410.73	$C_{20}H_{50}$	Alkene	45.171	0.78
32	Olean-12-en-3-One	424.713	$C_{30}H_{48}O$	Ketone	46.269	0.91
33	Pentacosane	352.691	$C_{25}H_{52}$	Alkane	47.341	4.37
34	Stigmasta-3,5-Dien-7-One	410.686	$C_{29}H_{46}O$	Ketone	49.179	0.78
35	Cholesta-4,6-Dien-3-Ol,Benzoate,(3-Beta)-	384.6377	$C_{27}H_{44}O$	Alcohol, ester	50.427	0.53
36	Cholest-5-ene, 3-Ethoxy-,(3-Beta)	386.664	$C_{27}H_{46}O$	Alkene, ester	51.431	0.56
37	Vitamin E	430.7061	$C_{29}H_{50}O_2$		51.821	0.68

Table 3: List of chemical compounds characterised in the leaf sample of *M. flavescens* through GC-MS analysis

Sl. no	Leaf sample	Molecular Weight g/mol	Molecular formula	Compound group	Rt	Peak area %
1	2(5H)-Furanone, 3-Methyl-5-Methylene	110.111	$C_6H_6O_2$	Ketone	7.418	33.82
2	5-Hydroxymethylfurfural	126.111	$C_6H_6O_3$	Aldehyde	13.530	6.92
3	2,3-2H-4-Methyl-Imidazole-2-One	98.105	$C_4H_6N_{20}$	Amine	16.264	4.50
4	6-Oxa-Bicyclo(3.1.0)Hexane-3-One	98.101	$C_5H_60_2$	Ketone	16.860	0.78
5	4-Methyl-2, 5-Dimethoxybenzaldehyde	180.203	$C_{10}H_{12}O_3$	Aldehyde	21.573	0.56
6	Dodecanoic Acid	200.3178	$C_{12}H_{24}O_2$	Carboxylic acid	21.761	1.46
7	TetradecanoicAcid	228.3709	$C_{14}H_{28}O_2$	Carboxylic acid	26.181	1.10
8	Vincadifformine	338.451	$C_{21}H_{26}N_2O_2$	Alkaloid	26.645	0.71
9	Neophytadiene	278.5157	$C_{20}H_{38}$	Alkene	27.709	6.25
10	2-Pentadecanone,6,10,14-Trimethyl	268.485	$C_{18}H_{36}O$	Ketone	27.795	1.13
11	Phytol,Acetate	338.5677	$C_{22}H_{42}O_2$	Phytol	28.197	1.07
12	9-Eicosyne	278.524	$C_{20}H_{38}$	Alkyne	28.575	2.08
13	Hexadecanoic Acid, Methyl Ester	270.4507	$C_{17}H_{34}O_2$	Fatty acids	29.485	1.12
14	DibutylPhthalate	278.348	$C_6H_4(COOC_4H_9)_2$ OR $C_{16}H_{22}O_4$	Carboxylic	30.056	4.04
15	Hexadecanoic Acid	256.43	$C_{16}H_{32}O_2$	Carboxylic	30.416	14.08
16	Mome Inositol	180.1559	$C_6H_{12}O_6$	Phenol	30.642	3.01

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 4, pp: (279-290), Month: October - December 2018, Available at: www.researchpublish.com

17	9-Octadecenoic Acid (Z)-,Methyl Ester	296.4879	$C_{19}H_{36}O_2$	Ester	32.812	1.09
18	9-Octadecenoic Acid	282.468	$C_{18}H_{34}O_2$	Carboxylic	32.911	0.62
19	Phytol	296.539	$C_{20}H_{40}O$	Fatty acids	33.016	3.11
20	5-Alpha-Pregnan-3. Beta –Amine, N,N,-Dimethyl-	331.588	$C_{23}H_{41}N$	Amines	33.558	2.48
21	Cis-VaccenicAcid	282.468	$C_{18}H_{34}O_2$	Carboxylic	33.665	4.31
22	OctadecanoicAcid	284.484	$CH_3(CH_2)_{16}COOH$ or $C_{18}H_{36}O_2$	Carboxylic	34.063	1.39
23	HexadecanoicAcid,2-Hydroxy-1-(Hydroxymethyl)Ethyl Ester	330.509	$C_{19}H_{38}O_4$	Ester	39.786	1.06
24	Squalene	410.73	$C_{20}H_{50}$	Alkene	45.153	0.60
25	Heneicosyl Heptafluorobutyrate	508.5965	$C_{25}H_{43}F_7O_2$	Carboxylic	47.284	0.90
26	Cholesta-4,6-Dien-3-Ol, Benzoate, (3 Beta)	384.6377	$C_{27}H_{44}O$	Ester	50.421	0.98
27	Vitamin E	430.7061	$C_{29}H_{50}O_2$		51.806	0.84

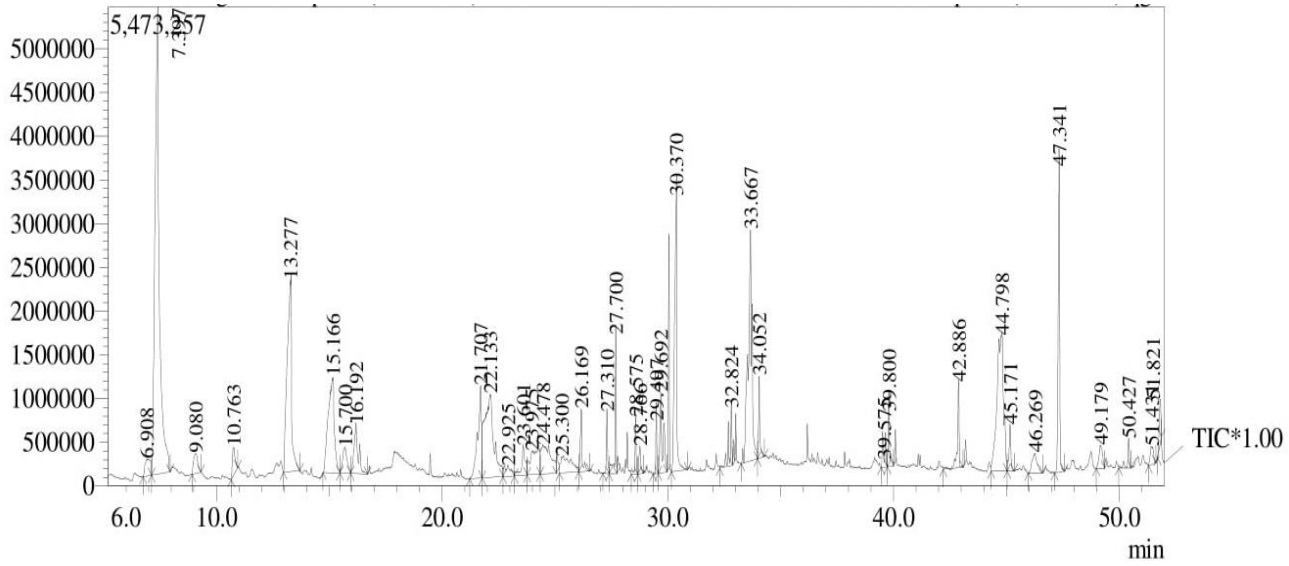

Fig 2: The GC-MS Chromatogram of methanolic extract of stem of *Memecylon flavescens*

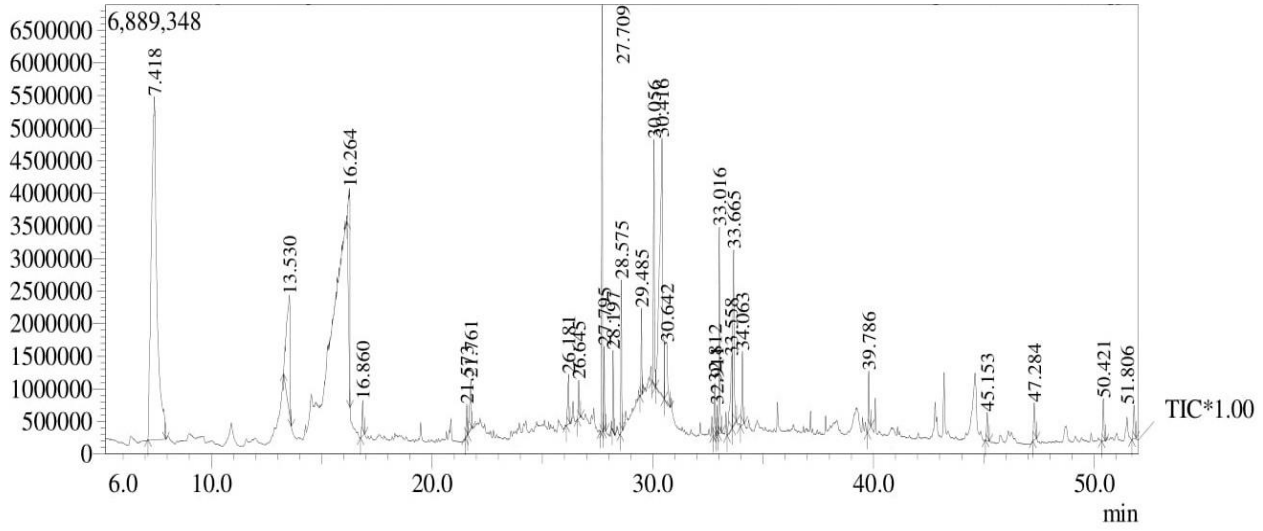

Fig 3: The GC-MS Chromatogram of methanolic extract of leaf of *Memecylon flavescens*

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 4, pp: (279-290), Month: October - December 2018, Available at: www.researchpublish.com

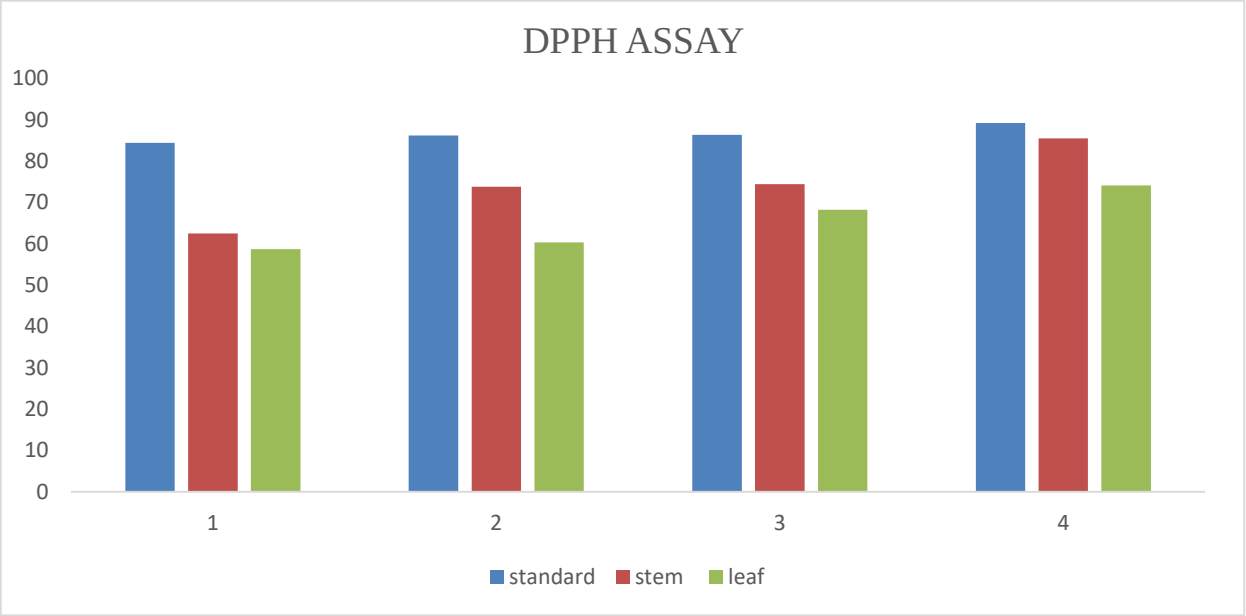

Fig 4: DPPH radical scavenging activity of methanolic extracts of leaf and stem of *Memecylon flavescens*.Standard-Ascorbic acid

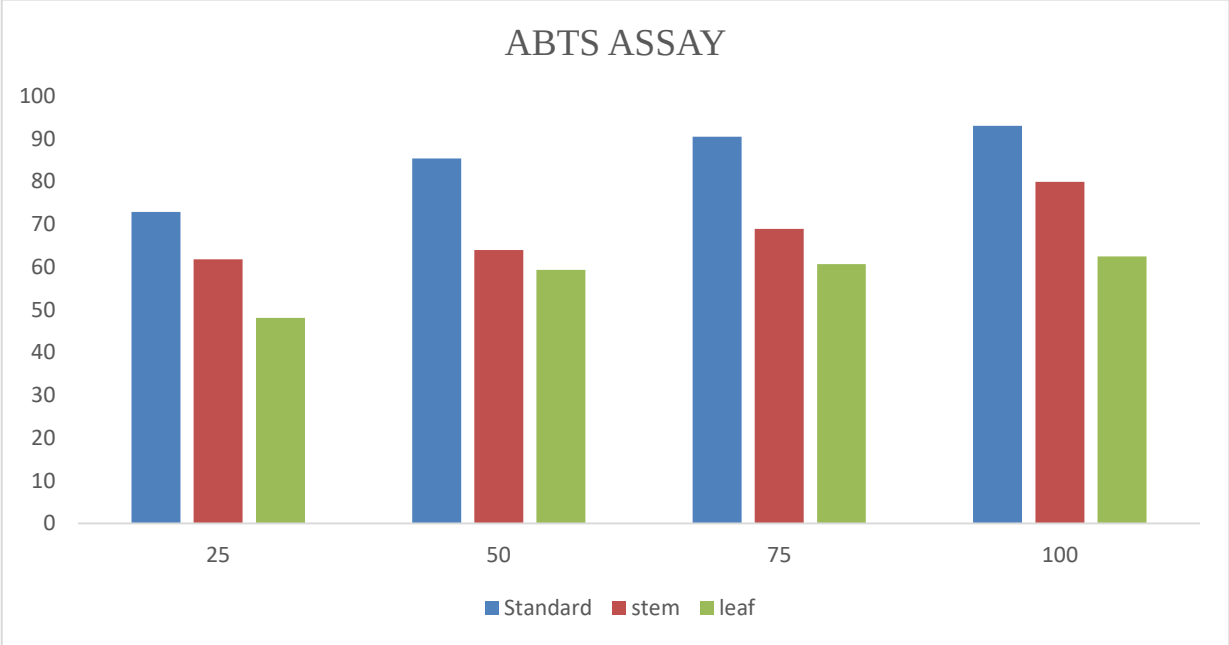

Fig 5: ABTS radical scavenging activity of methanolic extracts of stem and leaf of *Memecylon flavescens*.Standard-Ascorbic acid