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# Comparative seed morphology, pharmacognostic, phytochemical and antioxidant potential of *Memecylon* L. fruits.

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**ABSTRACT**
The present study unveils the seed morphology, pharmacognostic, phytochemical and antioxidant potential of *Memecylon* fruits. The species *viz.*, *M. grande* Blume*, M. randerianum* S. M & M. R. Almeida and *M. umbellatum* Burm. f. are selected for the study. *Memecylon* is the genus of the family Melastomataceae and their identification becomes difficult due to the intraspecies morphological similarities. So the identification of species becomes much strenuous. The surface morphology of seeds or fruits, pharmacognostic evaluation and phytochemical analysis are the effective methods to rectify the taxonomic difficulties in the authentification process and it opens a platform for the pharmaceutical analyses. The surface morphology of seeds was analyzed by scanning electron microscope and found that there is a distinct pattern of topography shown by the fruits of these plants. Scanning electron microscopic studies coupled with energy dispersive X-ray (EDX) analysis gave an insight on the elemental composition of the seeds. Inductively coupled plasma mass spectrometry is another technique used for evaluating elemental proportions of the fruits in parts per million units. These two analyses notify the presence of biologically potent trace elements in the fruit samples. While evaluating the pharmacognostic property of the seeds, powder microscopic analysis was found to be the prime and simple technique. This analysis remarks on the functional purity of the fruit samples. Hence it can be used for the formulation of the drugs in future. Quantitative estimation of secondary metabolites like alkaloids, terpenoids, flavonoids and phenols revealed that fruit extracts possess a potential amount of these phytoconstituents. The present study also reveals the antioxidant potential of *Memecylon* fruit extracts.
Keywords: *Memecylon,* scanning electron microscope, EDX, powder microscopy, secondary metabolites, antioxidants.

## INTRODUCTION

Green technology and alternative eco-friendly products are a brand new thought to several [1].The new lifestyle changes cause many perilous drawbacks, which opens a gateway for the

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search of new resolves. Thus nowadays the term "Green" becomes much popular. The major area under 'Green' consideration will be the medicinal field. Herbal medicines are a safe remedy for various human ailments because of it's less side effects and low-cost treatments. So there is wide acceptance of the herbal medicinal system. The quality measurements of herbs are a challengeable stream, where the validations of herbs are more important prior to the usage. Adulterations become a curse in the herbal medicinal field, they make inconsistent quality and safety. This will open a new approach to validate the quality assurance of herbs. The collection of plant materials, authentification of specimens, analysis and formulation of drugs is the way to the discovery of the safer natural drugs. Here an attempt was done for the evaluation of pharmacognostic characters of the medicinally important genus *Memecylon* fruits.

*Memecylon* is the genus of the family Melastomataceae. There are 289 species of shrubs and trees widely distributed in tropical regions. In India about 40 species are reported and 21 among them are endemic to the country.[2] *Memecylon* species are complex to identify because of their morphological similarities are confusing one. ***M. umbellatum*** and ***M. randerianum*** are common species found in the Western Ghats. Another selected species for the present study is ***M. grande*** which is present in the Western Ghats and dry deciduous forests. Previous reports reveal that these three species of *Memecylon* have potent medicinal activity [3,4]. ***M. umbellatum*** having elliptic-lanceolate leaves with umbellate inflorescence and yellow berries. ***M. randerianum*** possesses ovate-oblong leaves and blueberries and ***M. grande*** has ovate-lanceolate leaves with brownish blackberries.

Many systematic studies and new records are available on the genus *Memecylon*, but evaluations of micromorphological characters are trivial. Scanning electron microscopic analysis is the best way to analyze the surface features of the samples. The applications of SEM in vegetative and reproductive organs have great importance and impact on the systematic studies.[5]The functional purity of the plant sample is essential for the pharmaceutical trails. In this present study purity of the sample was analyzed through the powder microscopy, SEM-EDX and ICPMS techniques. Powder microscopy act as a diagnostic tool for the proper authentication of plant material.[6] SEM-EDX and ICPMS are effective methods for the qualitative and quantitative analyses of metal nano and microparticles in food products, plant or environmental.[7] The backbone behind the performance of the plants always hinge on the presence of bioactive metabolites. The majority of the pharmaceutical studies, phytochemical analysis is a curious step. Preliminary phytochemical analysis gave an insight on the phytochemical constituents present in the plant extracts. It comprises of qualitative and quantitative analysis. It gives valid information regarding the presence or absence of bioactive compounds in plant species. The plethora of biochemicals contribute to the specific bioactivity of plants, such as antimicrobial, antioxidant and anticancerous properties *etc*[8]. The present study also emphasises the evaluation of antioxidant potential of *Memecylon* fruit extracts. Antioxidants are free radical scavengers that can neutralize the oxidative stress induced by the Reacive Oxygen Species (ROS). Otherwise ROS can disrupt cellular mechanism and lead to severe pathological conditions and diseases like cancer, neurological disorders, atherosclerosis, hypertension, ischemia and diabetes[9]. The main objectives of this work are to assess the value and utility of the micromorphological typology and functional purity of the fruit samples as an additional source of evidence in the diagnosis and taxonomy of *Memecylon.*

## MATERIALS AND METHODS

### Plant material

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Fruits of *M. grande, M. randerianum* and *M. umbellatum* were selected for the present study. Ripened mature fruits of three species are collected from various parts of Kerala, India. Collected fruits were identified with the help of Dr. A. K. Pradeep Assistant Professor, Angiosperm Taxonomy Division, Department of Botany, University of Calicut, Kerala.

**SEM analysis**

Scanning electron microscopic (SEM) analysis was performed using the ZEISS Gemini SEM 300 machine. The samples were prepared on a carbon-coated copper grid. The technical features of Gemini SEM 300 are given below.

Gemini SEM 300 with resolutions: 0.6 nm @ 30 kV (STEM), 0.7 nm @ 15 kv, 1.2 nm @ 1 kv and 1.1 nm @ 1kV TD. Inlens BSE resolution: 1.2 nm @ 1 kV. Resolution in variable pressure mode (30 Pa): 1.4 nm @ 3 kV and 1.0 nm @ 15 kv. Acceleration voltage: 0.02 - 30 kV. Probe current: µ3 pA-20 Na. Magnification: 12 - 2,000,000.

**SEM EDX analysis**

SEM EDX analysis was done by using Octane plus with Gemini 300/EDS. The active area selected for the present study is 30 $mm^2$.

**Powder microscopy**

The fruit powder of *Memecylon* was analyzed under the bright field microscope for powder characteristics. The powder of the sample was treated with 4% KOH and mounted in glycerine on clean slides and the powder characters were photographed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss AxioCam Erc5s digitalcamera.

**ICP-MS analysis**

Inductively coupled mass spectrometric analysis was performed by using Agilent 7800 ICP-MS with Integrated Sample Introduction System (ISIS 3) and the SPS 4 autosampler. The standard torch of 2.5-mm-diameter injector and the instrument used a Ni sampler and Ni skimmer cones.

**Preliminary quantitative phytochemical analysis**

**Total alkaloid content**

The total alkaloid content was determined by the method of Shamsa et al.[10] The plant sample of 1 mg was dissolved in 2 N HCl and filtered. Add 5 ml phosphate buffer (pH 4.7), 5 ml BCG solution and shake the mixture with 1, 2, 3 and 4 ml of chloroform. The chloroform layer containing alkaloids was separated. Caffeine is used as a standard. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without sample. The total alkaloid content of the extract was calculated and expressed as mg caffeine equivalents (CE).

**Total flavonoid content**

Total flavonoid content was measured by the aluminium chloride colorimetric assay.[11] 1ml of extracts or standard solutions of quercetin (20, 40, 60, 80 and 100µg/ml) was added to a 10 ml volumetric flask containing 4 ml of distilled water. 0.30 ml of 5% $NaNO_2$ was added to the flask and after five minutes 0.3 ml 10 % $AlCl_3$ was added. After five minutes, 2 ml 1M NaOH was added and the volume was made up to 10 ml with distilled water. Gently mix the solution and read the absorbance at 510 nm against the blank solution. The total flavonoid content of the extract was calculated and expressed as mg quercetin equivalents (QE).

**Total phenolic content**

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Folin Ciocalteu assay was used for the determination of the total phenolic content of the sample solutions.[12] 1 ml of extracts was added to 25 ml of the volumetric flask containing 9 ml of distilled water. 1 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 minutes 10 ml of 7% $Na_2CO_3$ solution was added to the mixture. After an incubation period of 90 minutes at room temperature, the absorbance against the reagent blank was determined at 550 nm with an UV-Visible spectrophotometer. Gallic acid was used as standard and total phenolics content was expressed as mg Gallic acid Equivalents (GAE).

**Total terpenoid content**

Total terpenoid content of the plant extract was estimated by the methods of Ghori et al. [13] The reaction mixture contains aliquot of extract along with few drops of chloroform and $H_2SO_4$. The absorbance is measured at 538 nm against the blank. Linalool is used as a standard. The total terpenoid content of the extract was calculated and expressed as mg linalool equivalents (QE).

**Antioxidant activity**

The antioxidant activity of *Memecylon* fruits was determined on the basis of free radicals produced by various substrates like DPPH, $Fe^{3+}$ - ascorbate - EDTA - $H_2O_2$ system, sodium nitroprusside and potassium ferricyanide.

**DPPH free radical scavenging assay**

The DPPH free radical scavenging activity of *Memecylon* fruit extract was carried out by using the method of Chang et al.[14]. DPPH is a free radical, which reacts with antioxidant agents and gets reduced to DPPH-H. The pink coloured DPPH turns yellow when scavenged by antioxidants. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts. Ascorbic acid (10mg/ml DMSO) was used as a reference compound. Different volumes of extracts *viz.* 1.25µl - 20µl (12.5 - 200µg/ml) from a stock concentration of 10mg/ml were made up to a final volume of 20µl with DMSO and 1.48ml DPPH (0.1mM) solution was added. A control without the test compound, but an equivalent amount of distilled water was taken. The reaction mixture was incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control.

$$\% \text{ inhibition} = \frac{control - test}{control} X100$$

**Hydroxyl free radical scavenging activity**

Hydroxyl free radical scavenging activity was performed by the method of Kunchandy and Rao[15]. Different concentration of samples such as 125-2000µg/ml from a stock concentration of 10mg/ml was mixed with 500µl reaction mixture [2-deoxy 2-ribose (2.8mM), $FeCl_3$ (100µm), EDTA (100µm), $H_2O_2$ (1.0mM), ascorbic acid (100µm) in $KH_2PO_4$ - KOH buffer (20 mM pH 7.4)] was made up to a final volume of 1 ml. A control without the test compound, but an equivalent amount of distilled water was taken. After incubation for 1hour at 37°C, add 1ml of 2.8% TCA, then 1ml 1% aqueous TBA was added and the mixture was incubated at 90°C for 15 minutes to develop the colour. After cooling the absorbance was measured at 532 nm against an appropriate blank solution. Here gallic acid (10mg/mL DMSO) was used as reference.

$$\% \text{ inhibition} = \frac{control - test}{control} X100$$

**Nitric oxide free radical scavenging activity**

The determination of nitric oxide free radical scavenging activity of *Memecylon* fruit extracts was performed by using the method of Kumaran and Karunakaran [16]. Sodium nitroprusside ($5mmolL^{-1}$) in phosphate buffered saline solution (pH 7.4) was mixed with different

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concentration of the extracts *viz.* 125-2000µg /ml from a stock solution. It is incubated at 25°C for 30minutes. A control without the test compound, but an equivalent amount of distilled water was taken. After 30minutes, 1.5mL of the incubated solution was removed and diluted with 1.5mL of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1-naphthylethylenediamine dihydrochloride). The absorbance was measured at 546 nm and the percentage scavenging activity was measured with reference to the standard. Here gallic acid (10mg/mL DMSO) was used as reference.

% inhibition = $\frac{control-test}{control} X100$

**Super oxide free radical scavenging activity**

Super oxide free radical scavenging activity was carried out by using the method of Valentão et al.[17] Different concentration of sample such as 125 - 2000µg/ml prepared from a stock solution of 10mg/ml, 0.05ml of Riboflavin solution (0.12mM), 0.2 ml of EDTA solution (0.1M), and 0.1 ml NBT (Nitro blue tetrazolium) solution (1.5mM) were mixed in a test tube and reaction mixture was diluted up to 2.64ml with phosphate buffer (0.067M). A control without the test compound, but an equivalent amount of distilled water was taken. The absorbance of solution was measured at 560 nm after illumination for 5 minutes incubation in fluorescent light and also measured after illumination for 30 min. at 560 nm on UV visible spectrophotometer. Ascorbic acid (10mg/ml DMSO) was used as reference.

% inhibition = $\frac{control-test}{control} X100$

## RESULTS AND DISCUSSION

Scanning electron microscopy is a method for high resolution surface imaging using an electron beam having greater magnification and much larger depth of field. The seed capsule micromorphology and the entire seed morphology were studied by using a scanning electron microscope. The difference in electron emission in different areas provides the surface topography of the material. In this study, all the selected species show distinct morphological patterns. The seed surface characteristics often provide valuable assistance in delimiting generic and taxonomic relationships.[18] SEM analysis of *Memecylon* fruits is a novel report. In the case of *M. grande,* fruits show colliculate pattern with a 6.2 mm seed capsule and the seed surface possesses tuberculate pattern with a 5.8 mm width (**Figure 1 A1, B1, C1, D1**). Scanning electron microscopic technique reveals that the seed capsule of *M. randerianum* has a pattern of ruminate reticulate type. (**Figure 1 A2, B2, C2, D2**). The width of the capsule was 4.2 mm. The seed surface of *M. randerianum* is of a reticulate pattern and has 3.5 mm width. *M. umbellatum* seed capsule possesses a smoothened pattern having a 5.6 mm width and its seed surface shows a wrinkled pattern with a 3.81 mm width (**Figure 1 A3, B3, C3, D3**). The characteristic surface morphology becomes a useful tool in the identification process.

Energy dispersive X-ray microanalysis (EDX) is a technique for analyzing elemental composition at the microscopic level. For this purpose, scanning electron microscope (SEM) is equipped with an energy dispersive system having a quantitative electron probe for X-ray microanalysis. The SEM-EDX system can be applied to surfaces of untreated specimens and, thus provides a vivid picture of elemental distributions in plant and animal material[19]. The elemental composition of *M. grande* fruits show that nitrogen content becomes 91% and other elements like phosphorus 3.10%, potassium 1.53%, iron 1.41%, magnesium 0.87% and sodium 0.55% (**Figure 2**). In the case of *M. randerianum* fruit, nitrogen is the prominent element with

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93% of the weight. Copper 0.49, cobalt 0.90, zinc 0.09, sodium 0.03, magnesium 0.22, phosphorus 4.01, potassium 1.15 and calcium 0.09% are the revealed composition of other elements (**Figure 3**). *M. umbellatum* fruit also possesses an elevated amount of nitrogen (93%) and all other elements in trace amount. Phosphorus 3.4%, potassium 1%, copper 0.95%, magnesium 0.67%, cobalt 0.34%, and iron 0.22% (**Figure 4**). This finding offers that *Memecylon* fruits are a reservoir of essential elements and it can be exploited in the pharmaceutical or nutritional field.

In Ayurveda, 90% of the preparations are plant-based, hence the worthwhile usage of medicines are promising candidates for the remedies of various human ailments. In most of the Ayurvedic preparations, the powdered samples of plant parts are used. So the authenticity of the powdered sample is very important. Powder microscopy is a simple and easiest method to analyze the powder sample and it is an essential step in the pharmacognostic evaluation of the plant sample. Microscopic techniques examine the structural and cellular features of herbs to determine their botanical origin. Microscopic evaluation is now an indispensable tool for the identification of medicinal herbs and is one of the important parameters in modern.[20] Here the powder samples of *Memecylon* fruits were characterized through their microscopic characters. The powder of *M. grande* fruits was brown coloured, odourless and slightly astringent (**Figure 5**). The characters found in the powders are epicarp cells, parenchyma cells with starch grains from mesocarp, stone cells from mesocarp, sclereids from endocarp, vessels with spiral and annular thickenings and rosette crystals. In the case of *M. randerianum* fruits powder, it is brown coloured, odourless with a characteristic taste (**Figure 6**). It contains epicarp cells, mesocarp parenchyma cells, stone cells, sclereids from endocarp, tracheids, fiber bundles and rosette crystals. The same brown colored powder has also occurred in the *M. umbellatum* fruits (**Figure 7**). Powder showed characters like epicarp cells, pitted parenchyma cells from mesocarp, stone cells, sclereids, spiral vessels, fibro-sclereids and rosette crystals. These characters can be used to identify the authenticated plant specimen in the Ayurvedic preparations. So we can easily identify the botanical origin of the plant specimen and clearly distinguish the presence of adulterants or the allied species. The microscopic evaluation of *M. umbellatum* leaves was done by Killedar et al. [6]and found the presence of lignified xylem with well-defined xylem fibers, vessels, and parenchyma. The presences of phloecentric vascular bundles surrounded by endodermis and crystal sheath were also reported. The powder microscopic analysis confirms that the botanical origins of the plant samples are pure and devoid of foreign particles. So this result can be used as future reference for identification of *Memecylon* fruits.

During the past decades, we all have very much care about the nutritional status of our body. We got an insight into the profound effect of micro and macronutrients on biological processes that range from whole-organism performance to the cellular function. According to the classification of trace elements, the group I which include carbon, hydrogen, oxygen, and nitrogen are the basic components of macromolecules such as carbohydrates, proteins, and lipids. Group II category includes nutritionally important minerals such as sodium, potassium, chloride, calcium, phosphorous, magnesium and sulfur. In group III, some essential trace elements like copper, iron, zinc, chromium, cobalt, iodine, molybdenum, and selenium are found[21]. Copper plays an important role in metabolism mainly in the proper functioning of the enzymes and its deficiency may cause hypochromic anemia, joint pain neutropenia, hypopigmentation of hair and skin, abnormal bone formation with skeletal fragility and osteoporosis *etc.*[22] Another most important element is iron, which is a prime portion of the blood cells and its deficiency is called anemia. Anemia is the second most important cause of maternal mortality in India and 20% of mortality

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is directly related to anemia and another 50% is associated with other anemic side effects. In the case of zinc, it is essential for normal spermatogenesis and maturation, proper development of thymus, proper epithelialization in wound healing, taste sensation, and secretion of pancreas and gastric enzymes.[23]

In addition to SEM-EDX analysis, to substantiate the quality of the fruit samples in their elemental composition, Inductively Coupled Plasma Mass Spectrometry analysis was carried out. This technique gave the details of elements present in the sample in part per million units and determination of thirteen elements were done *ie.,* aluminum (Al), arsenic (As), cadmium (Cd), cobalt (Co), *strontium (Sr), selenium* (Se), chromium (Cr), copper (Cu), molybdenum (Mo), nickel (Ni), lead (Pb), barium (Ba) and manganese (Mn). *M. grande* fruits show a promising concentration of the majority of elements except for molybdenum and lead. Lead and molybdenum concentration were found to be higher in the case of *M. randerianum* fruit (**Table 1).** The standard reference concentrations of trace elements present in the adult human blood samples are noticed**,** because it is essential for the standardization of drugs**.** Most of the detected elements show vital biological functions**.** Some elements are the part of vitamins, cofactors of enzymes, oxidative phosphorylation, fatty acids and cholesterol metabolism. There are no known health benefits or the biological role of lead for the human body. It has been found that chromium produces significant increases in enzyme activity and serves as a stimulator in fatty acid and cholesterol biosynthesis from acetate molecule in the liver. It can also enhance sugar metabolism through the activation of insulin.[24] In the case of cobalt, it is the key factor of cobalamin (vitamin B12) and it has a role in the formation of amino acids and neurotransmitters. Although the biological function of nickel is still somewhat unclear in the human body, however nickel found in higher concentration in the nucleic acids, particularly RNA, and is thought to be involved in protein structure or function.[25] So the biological role of these trace elements is significant in regulating homeostasis and prevention of free radical damage and various human ailments [26].

Preliminary quantitative analysis of various phytochemicals was carried out by using standard protocols. *M. grande* fruit was found to be rich in alkaloids, flavonoids, phenolics and terpenoids (**Table 2**). All these phytoconstituents have a significant biological role. Liu [27] proposes that phytochemicals are non nutrient compounds that can reduce the risk of major non communicable chronic diseases and that are commonly found in fruits, vegetables, grains, and other plant foods. Alkaloids have a wide spectrum of pharmacological activity [28], it includes antifungal, antihyperglycemic, antityrosinase, antiglucosidase, antinociceptive and anti-inflammatory *etc*. The efficiency of bioactive natural products in curing pathological conditions of hyperlipidemia, atherosclerosis and hypertension was studied by Liwa et al.[29] Polyphenols and phenolic compounds from natural sources can improve vascular vasodilator function, and are protective against hypertension and CVDs. Flavonoids have anti-inflammatory, anticancerous and antimutagenic activities already being reported[30]. Tan et al.[31] confirms that the progressive usage of phytochemicals through diets as an effective method to cure the diseases. *Memecylon* fruit extract shows promising antioxidant activity in four different assays. All the fruit extracts show a dose dependent scavenging activity. Among the extracts, *M. grande* fruit extract shows highest free radical scavenging activity in all assays, followed by *M. umbellatum* and M. *randerianum* fruit extracts **(Figure 8, 9, 10, 11)**. *M. grande* fruit extracts shows highest scavenging activity in nitric oxide assay (76.85 ± 0.08) and least at hydroxyl radical scavenging assay (61.69 ± 0.56). *M. randerianum* fruit shows the least activity in hydroxyl radical scavenging assay (46.16 ± 0.17). Based on the four assays conducted, the most effective antioxidant extracts was that of *M.*

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*grande* fruit with an IC $_{50}$ of 83.9195 ± 0.14. This was followed by ***M. umbellatum*** with $IC_{50}$ of 91.1031 ± 0.12 and *M. randerianum* having 104.178 ± 0.13 **(Table 3)**. There is a significant relationship between antioxidant capacity and total phenolic content, indicating that phenolic compounds are the major contributors to the antioxidant potential of these plants[32]. The quantitative phytochemical analysis justifies the high performance of these species in the antioxidant assays. The highest phenolic content was found in the fruit extract of ***M. grande*** (370.28 ± 8.36). So it can be considered as a good candidate for pharmaceutical plant-based products.

**Study limitations**: The detailed phytochemical characterization and compound isolation can be more satisfactory in pharmaceutical applications. The present findings are useful in pharmaceutical field because the botanical purity of plants is the prime important step in natural drug formulation. Definitely, these results can be used as a future reference for *Memecylon* fruits evaluation.

## CONCLUSIONS

The seed surface characteristics are often a valuable support in delimiting generic and taxonomic relationships. Powder microscopy, SEM-EDX and ICPMS analysis have provided knowledge about the functional purity and elemental composition of the fruit samples. The free radical scavenging assays provides acquaintance of a natural antioxidant source. So these ample findings can be effectively targeted towards the pharmacological utility of *Memecylon* fruits.

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| . | | Concentration (ppm) | Reference |
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**Table 1:** ICPMS analysis of *Memecylon* fruits (1µg/L = 0.001 ppm)

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| Element | Mass | MGF | MRF | MUF | concentration of trace elements in adult human blood (µg/L)[20] |
|---|---|---|---|---|---|
| Al | 27 | 83135.864 | 41909.731 | 38739.426 | 2-8 |
| Cr | 52 | 10.223 | 5.923 | 4.016 | <5 |
| Mn | 55 | 1790.173 | 786.657 | 272.022 | 8-12 |
| Co | 59 | 1.658 | 0.627 | 0.709 | 5-10 |
| Ni | 60 | 46.030 | 9.316 | 30.256 | 1-5 |
| Cu | 63 | 191.231 | 69.677 | 85.967 | 800-1100 |
| Zn | 66 | 320.408 | 119.412 | 118.666 | 6000-7000 |
| As | 75 | 1.620 | 1.048 | 0.958 | 2-20 |
| [As] | 77 | 19.861 | 5.952 | 5.418 | 2-20 |
| [As] | 78 | 25.097 | 9.117 | 8.453 | 2-20 |
| Se | 82 | 14.152 | 2.834 | 0.337 | 90-130 |
| Sr | 88 | 880.178 | 148.725 | 275.348 | 1.5-3.9 |
| Mo | 95 | 0.843 | 18.423 | 3.921 | 1-3 |
| Cd | 111 | 1.013 | 0.364 | 0.304 | 0.3-1.2 |
| Ba | 137 | 451.711 | 111.353 | 105.197 | 0.5-2.5 |
| [Pb] | 206 | 24.184 | 58.300 | 17.053 | 50-150 |
| [Pb] | 207 | 24.904 | 59.807 | 17.365 | 50-150 |
| Pb | 208 | 24.558 | 63.138 | 17.170 | 50-150 |

**Table 2:** Preliminary quantitative phytochemical analysis of *Memecylon* fruits. SE: standard error. Means within a column followed by the same letters are not significantly different at $P < 0.05$ as determined by Duncan's multiple range test.

| Plants | Alkaloids (mg caffeine/g DW) ± SE | Flavonoids (mg quercetin/g DW) ± SE | Phenolics (mg GAE/g DW) ± SE | Terpenoids (mg linalool/g DW) ± SE |
|---|---|---|---|---|
| MGF | 52.16 ± 3.23 $^{d}$ | 91.77 ± 2.65 $^{c}$ | 370.28 ± 8.36 $^{d}$ | 378.21 ± 19.02 $^{c}$ |
| MRF | 32.17 ± 1.41 $^{a}$ | 21.40 ± 2.72 $^{b}$ | 276.06 ± 14.12 $^{c}$ | 355.03 ± 57.31 $^{c}$ |
| MUF | 36.47 ± 0.66 $^{ab}$ | 57.57 ± 4.40 $^{b}$ | 60.83 ± 5.70 $^{a}$ | 127.5 ± 10.50 $^{a}$ |

**Table 3**: The effect of methanolic extracts of *Memecylon* fruits in different antioxidant assays.

| IC 50 Values (µg/mL) | | | | |
|---|---|---|---|---|
| Plants | DPPH radical scavenging assay | Hydroxyl radical scavenging assay | Nitric oxide radical scavenging assay | Super oxide radical scavenging assay |
| Standard | 48.8412 ± 1.5$^{a}$ | 1347.51 ± 0.27$^{b}$ | 346.207 ± 0.01$^{a}$ | 238.357 ± 0.03 $^{a}$ |
| MGF | 83.9195 ± 0.14 $^{b}$ | 1231 ± 0.48$^{a}$ | 696.733 ± 0.06 $^{b}$ | 698.991 ± 0.03 $^{b}$ |

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| | | | | |
|---|---|---|---|---|
| MRF | $104.178 \pm 0.13^{d}$ | $2029.57 \pm 0.14^{e}$ | $1081.61 \pm 0.01^{d}$ | $1311.24 \pm 0.02^{e}$ |
| MUF | $91.1031 \pm 0.12^{c}$ | $1696.73 \pm 0.05^{c}$ | $916.988 \pm 0.04^{c}$ | $1129.34 \pm 0.01^{c}$ |

Means within a column followed by the same letters are not significantly different at $P < 0.05$ as determined by Duncan's multiple range test.

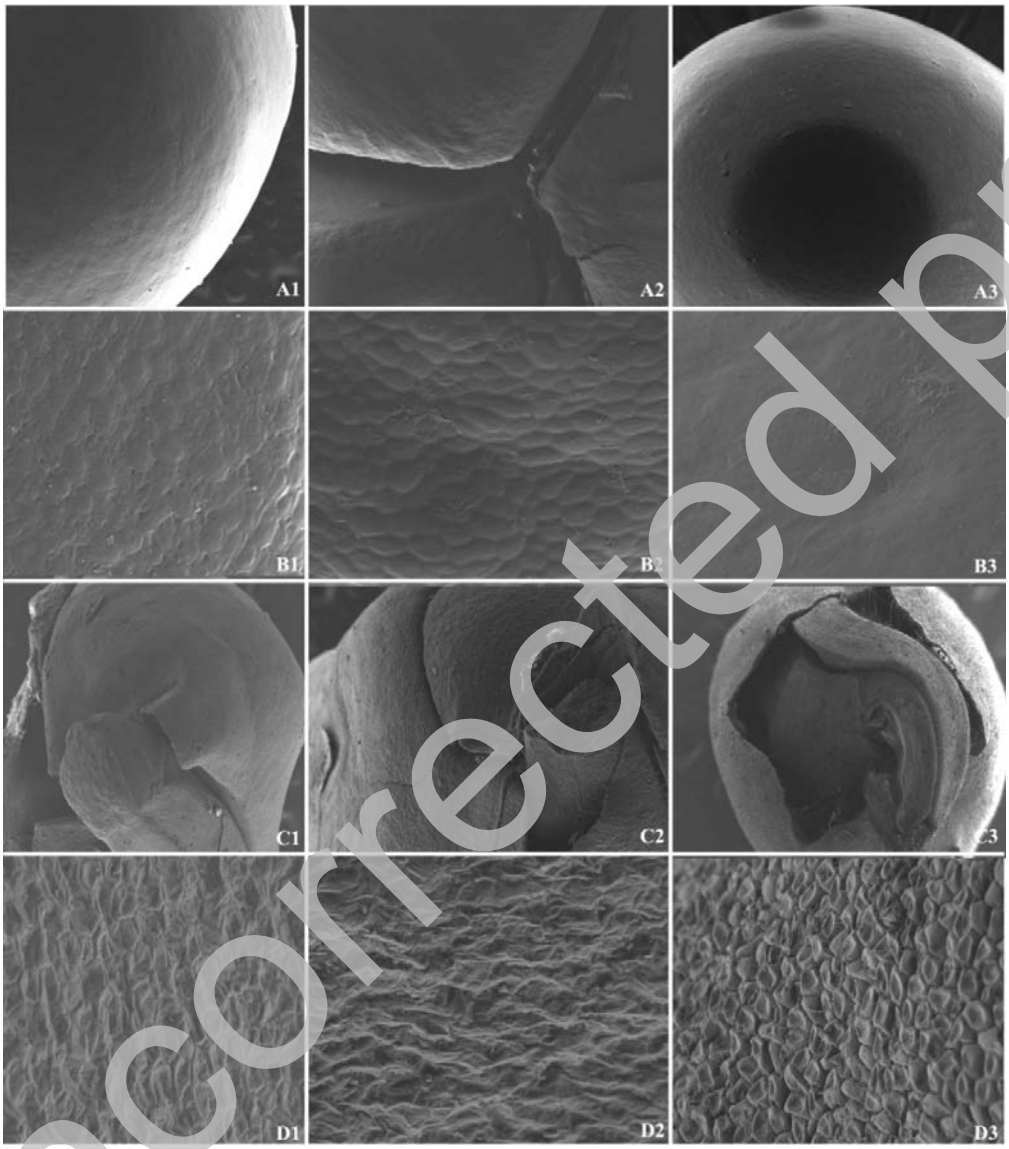


**Figure 1:** Scanning electron microscopic analysis *of Memecylon* fruits.
**A1** *M. grande* seed capsule, **B1** Enlarged view, **C1** seed surface, **D1** Enlarged view.
**A2** *M. randerianum* seed capsule, **B2** Enlarged view, **C2** seed surface, **D2** Enlarged view.
**A3** *M. umbellatum* seed capsule, **B3** Enlarged view, **C3** seed surface, **D3** Enlarged view.

**Figure 2: SEM – EDX analysis of *M. grande* fruits**

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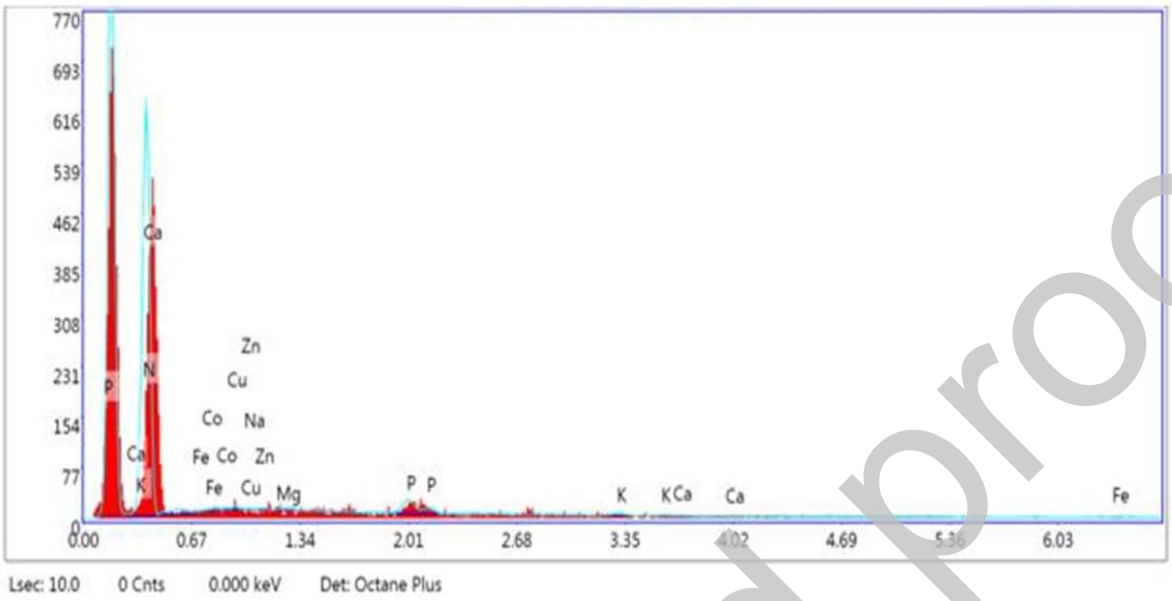


| Element | Weight % | Atomic % | Net Int. | Error % | Kratio | Z | A | F |
|---|---|---|---|---|---|---|---|---|
| N K | 93.07 | 97.14 | 352.96 | 5.86 | 0.7757 | 1.0137 | 0.8222 | 1.0000 |
| FeL | 0.22 | 0.06 | 0.27 | 99.99 | 0.0009 | 0.7345 | 0.5352 | 1.0000 |
| CoL | 0.34 | 0.08 | 0.58 | 99.99 | 0.0017 | 0.7179 | 0.6777 | 1.0000 |
| CuL | 0.95 | 0.22 | 2.47 | 87.46 | 0.0054 | 0.7059 | 0.8034 | 1.0000 |
| ZnL | 0.11 | 0.02 | 0.30 | 99.99 | 0.0007 | 0.7054 | 0.8850 | 0.9996 |
| NaK | 0.02 | 0.01 | 0.10 | 99.99 | 0.0001 | 0.8829 | 0.6657 | 1.0014 |
| MgK | 0.67 | 0.41 | 4.65 | 75.71 | 0.0048 | 0.8943 | 0.7964 | 1.0024 |
| P K | 3.40 | 1.61 | 17.74 | 29.96 | 0.0278 | 0.8363 | 0.9699 | 1.0066 |
| K K | 1.00 | 0.37 | 2.78 | 75.64 | 0.0082 | 0.7962 | 1.0058 | 1.0263 |
| CaK | 0.22 | 0.08 | 0.47 | 99.99 | 0.0018 | 0.8074 | 1.0069 | 1.0336 |

**Figure 3: SEM – EDX analysis of *M. randerianum* fruits**

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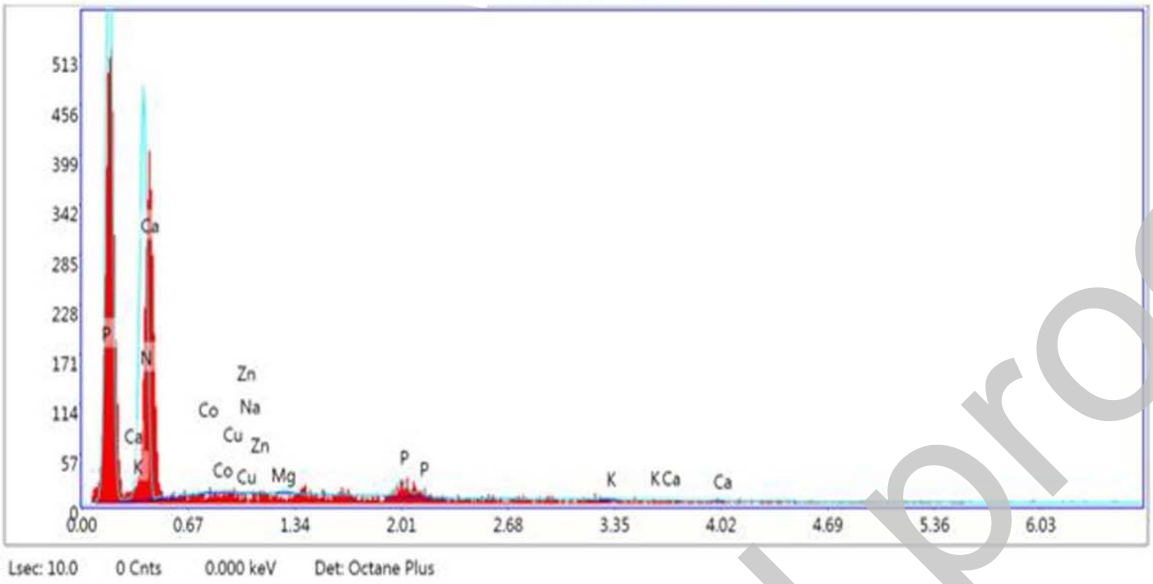


| Element | Weight % | Atomic % | Net Int. | Error % | Kratio | Z | A | F |
|---|---|---|---|---|---|---|---|---|
| N K | 93.02 | 97.14 | 265.63 | 6.41 | 0.7540 | 1.0139 | 0.7994 | 1.0000 |
| CoL | 0.90 | 0.22 | 1.14 | 92.04 | 0.0043 | 0.7180 | 0.6575 | 1.0000 |
| CuL | 0.49 | 0.11 | 0.95 | 99.99 | 0.0027 | 0.7060 | 0.7821 | 1.0000 |
| ZnL | 0.09 | 0.02 | 0.20 | 99.99 | 0.0006 | 0.7055 | 0.8732 | 0.9995 |
| NaK | 0.03 | 0.02 | 0.10 | 99.99 | 0.0002 | 0.8830 | 0.6427 | 1.0014 |
| MgK | 0.22 | 0.13 | 1.17 | 82.21 | 0.0015 | 0.8944 | 0.7806 | 1.0026 |
| P K | 4.01 | 1.89 | 16.70 | 30.68 | 0.0327 | 0.8365 | 0.9688 | 1.0065 |
| K K | 1.15 | 0.43 | 2.58 | 77.90 | 0.0095 | 0.7963 | 1.0061 | 1.0249 |
| CaK | 0.09 | 0.03 | 0.15 | 99.99 | 0.0007 | 0.8076 | 1.0071 | 1.0332 |

**Figure 4: SEM - EDX analysis *M. umbellatum* fruits**

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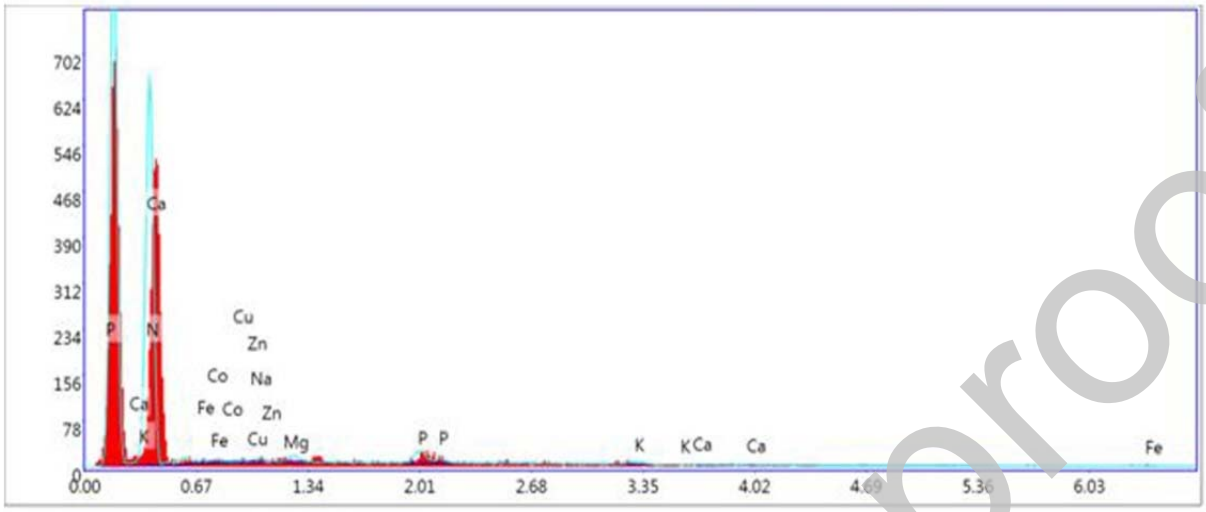


| Element | Weight % | Atomic % | Net Int. | Error % | Kratio | Z | A | F |
|---|---|---|---|---|---|---|---|---|
| N K | 90.98 | 96.26 | 371.42 | 6.21 | 0.7249 | 1.0182 | 0.7826 | 1.0000 |
| FeL | 1.41 | 0.37 | 1.99 | 77.04 | 0.0055 | 0.7379 | 0.5306 | 1.0000 |
| CoL | 0.63 | 0.16 | 1.18 | 99.99 | 0.0030 | 0.7212 | 0.6641 | 1.0000 |
| CuL | 0.48 | 0.11 | 1.38 | 99.99 | 0.0027 | 0.7092 | 0.7867 | 1.0000 |
| ZnL | 0.11 | 0.03 | 0.34 | 90.61 | 0.0007 | 0.7087 | 0.8740 | 0.9996 |
| NaK | 0.55 | 0.36 | 3.02 | 95.61 | 0.0032 | 0.8870 | 0.6530 | 1.0013 |
| MgK | 0.87 | 0.53 | 6.70 | 69.73 | 0.0061 | 0.8985 | 0.7839 | 1.0022 |
| P K | 3.10 | 1.48 | 18.42 | 29.52 | 0.0253 | 0.8403 | 0.9655 | 1.0070 |
| K K | 1.53 | 0.58 | 4.86 | 71.10 | 0.0126 | 0.8001 | 1.0055 | 1.0260 |
| Ca K | 0.35 | 0.13 | 0.86 | 90.39 | 0.0030 | 0.8114 | 1.0060 | 1.0326 |

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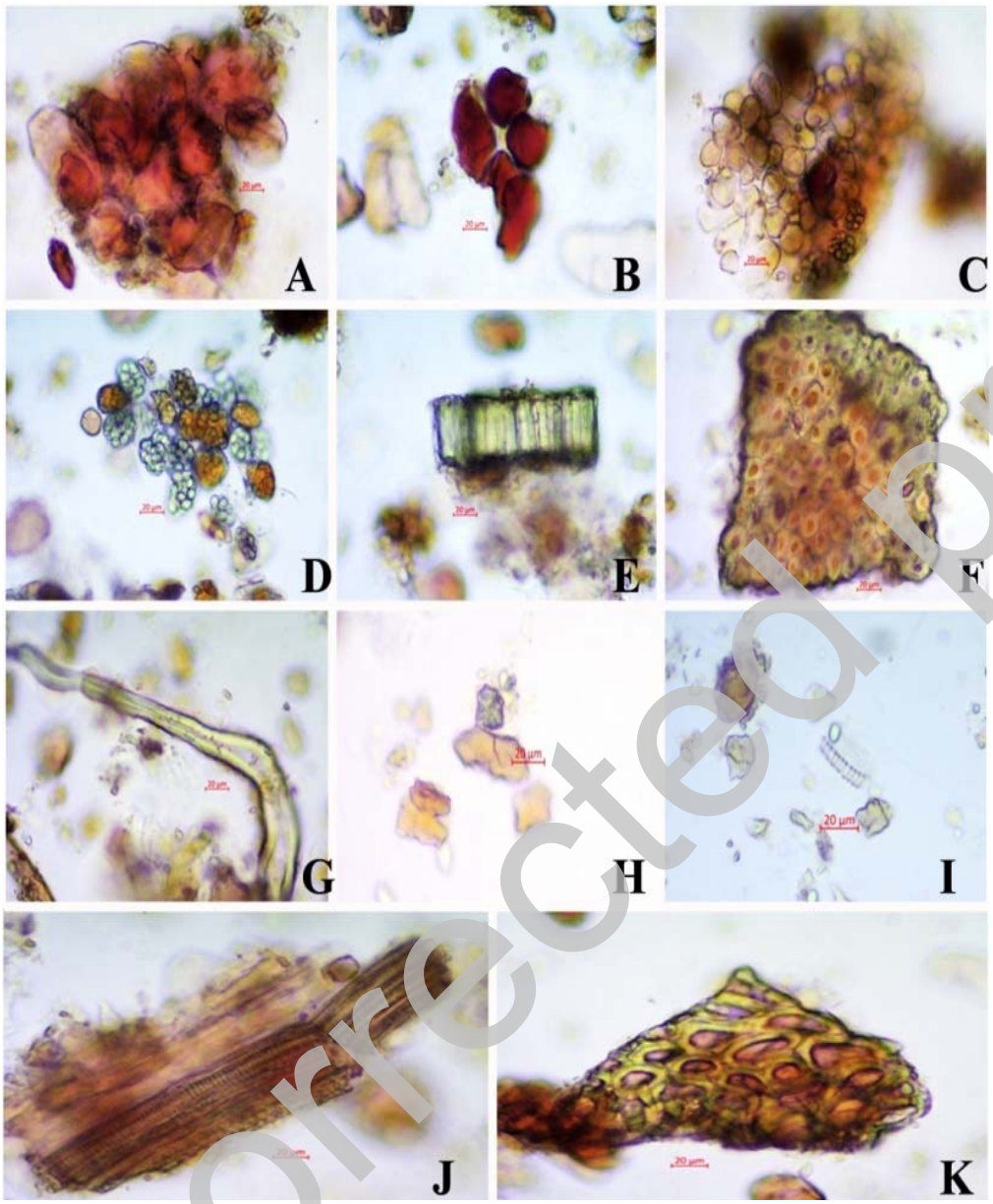


**Figure 5: Powder microscopic analysis of *M. grande* fruits. A, B** Epicarp cells; **C, D** Mesocarp parenchyma cells with starch grains; **E** Transversely cut testa; **F** Sclereidal fiber; **G** Rosette crystal; **H** Spiral vessels; **I** Sclereids from endocarp; **J** Annular vessels; **K** Testa in surface view.

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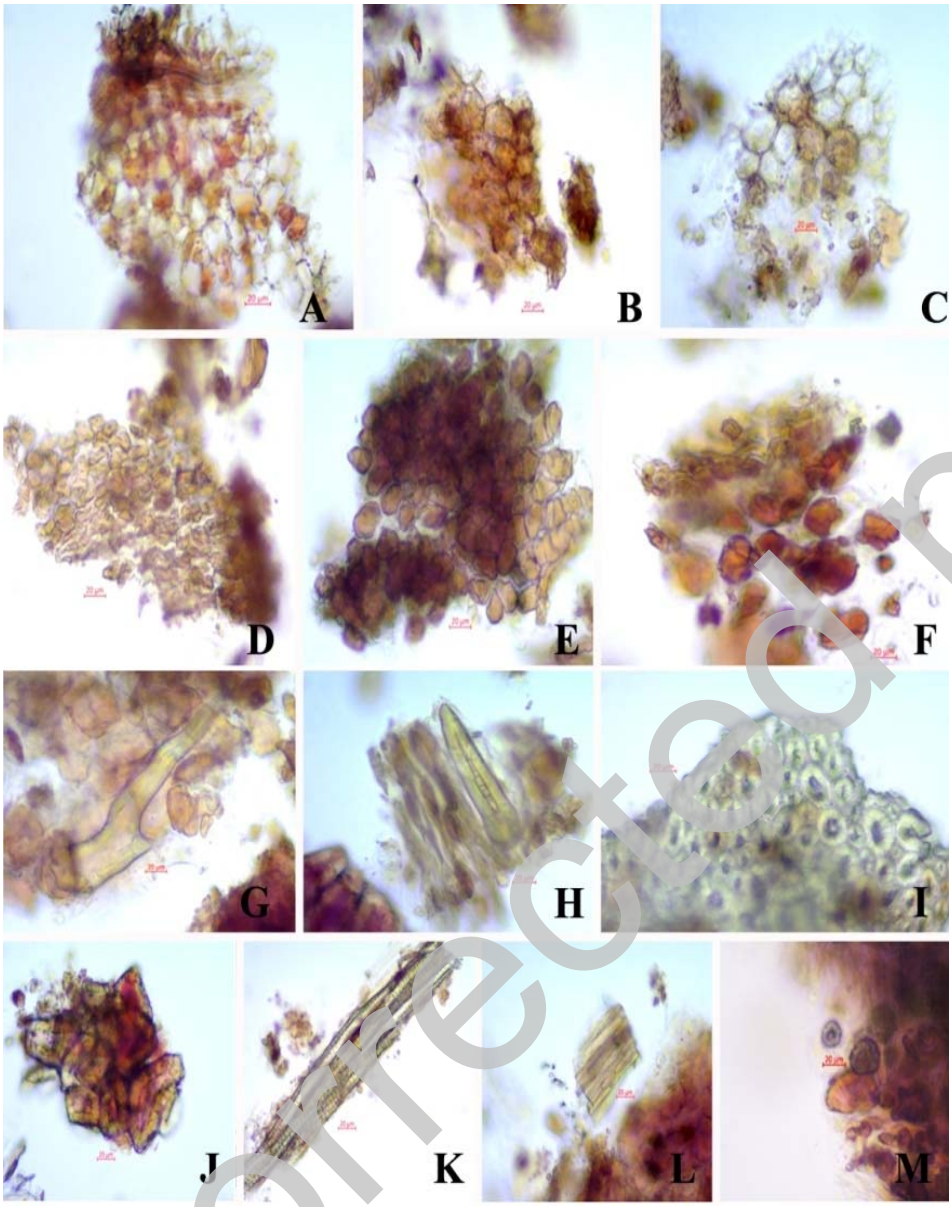


**Figure 6: Powder microscopic analysis of *M. randerianum* fruits.** **A** Mesocarp in sectional view; **B** Epicarp in surface view; **C, D, E, F** Mesocarp cells **G, H** Sclereids; **I, J** Stone cells; **K** Tracheids; **L** Fiber bundles; **M** Rosette crystals.

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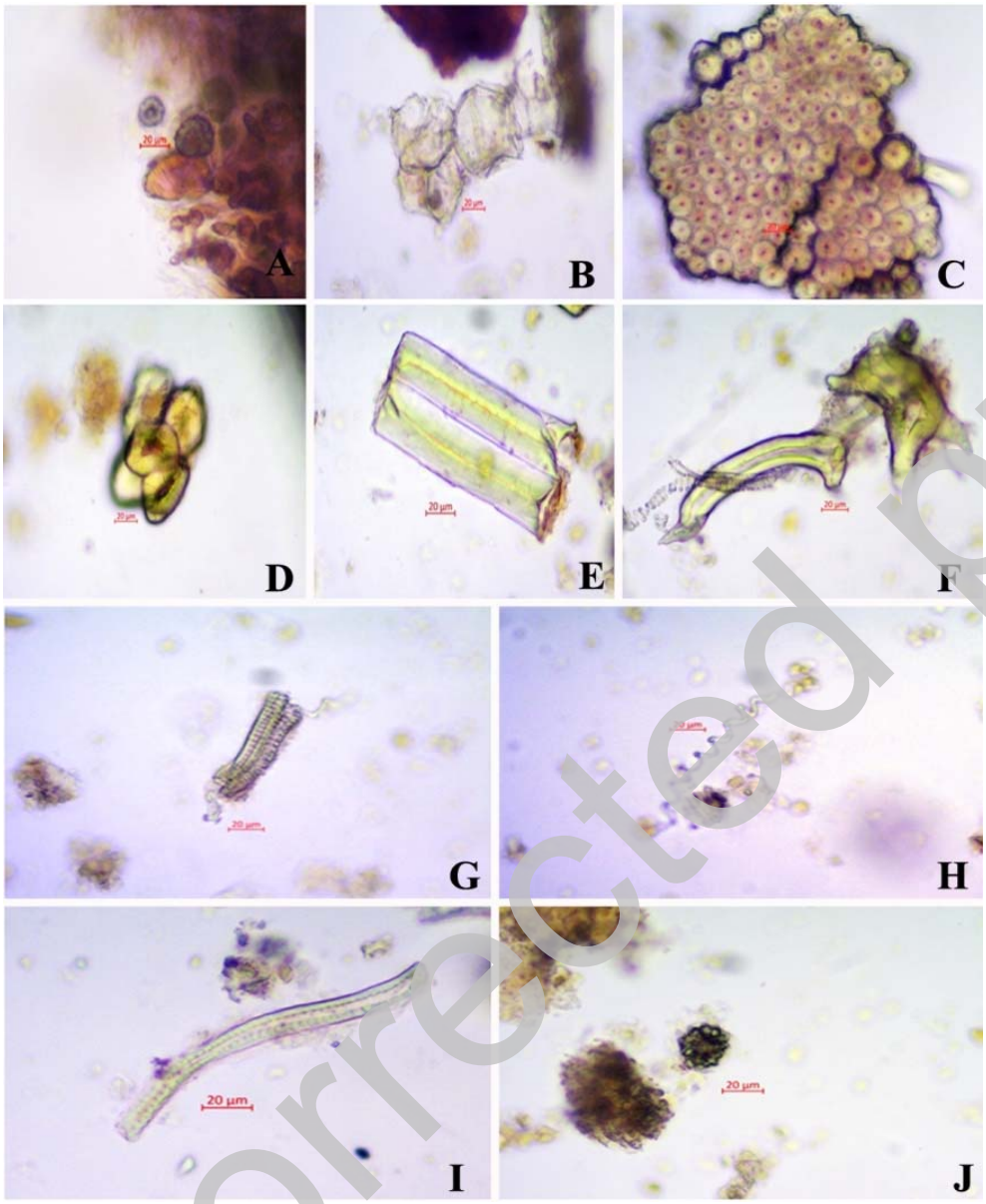


**Figure 7: Powder microscopic analysis of *M. umbellatum* fruits. A** Epicarp cells; **B** Pitted parenchyma cells of mesocarp; **C** Testa in surface view; **D** Stone cells; **E, F** Sclereids; **G, H** Spiral vessels; **I** Fibro-sclereid; **J** Rosette crystals.

**Figure 8**: *In vitro* DPPH scavenging activity of *Memecylon* fruits.

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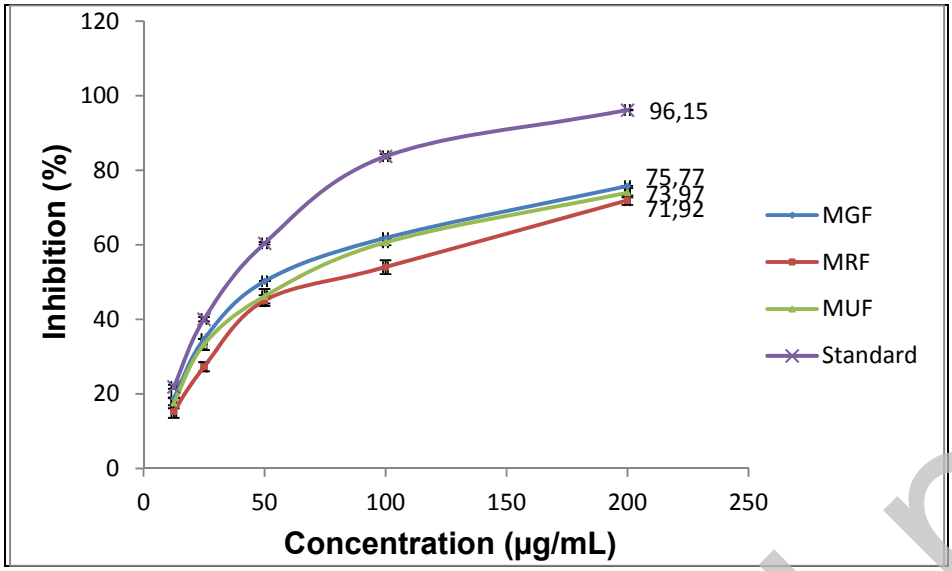


**Figure 9**: *In vitro* hydroxyl radical scavenging activity of *Memecylon* fruits

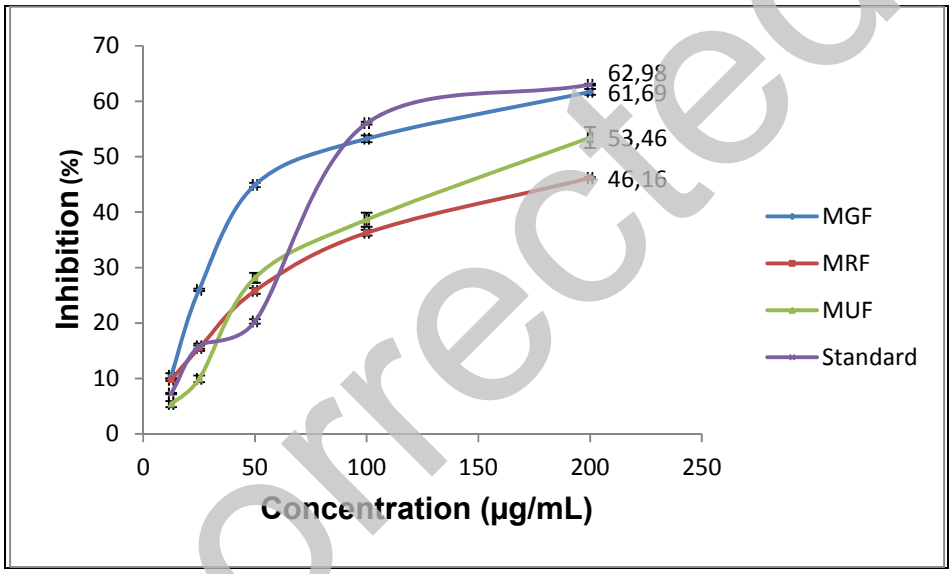


**Figure 10**: *In vitro* nitric oxide radical scavenging activity of *Memecylon* fruits

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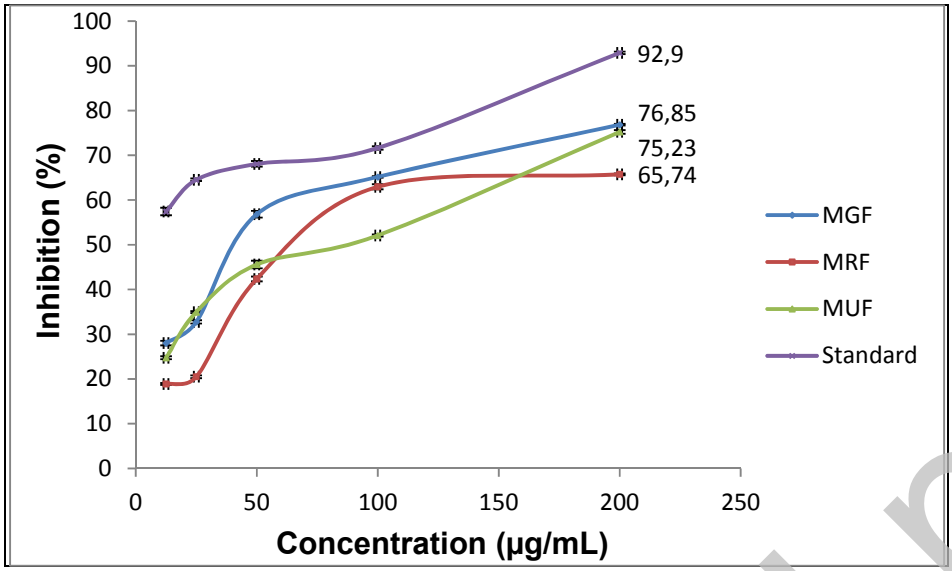
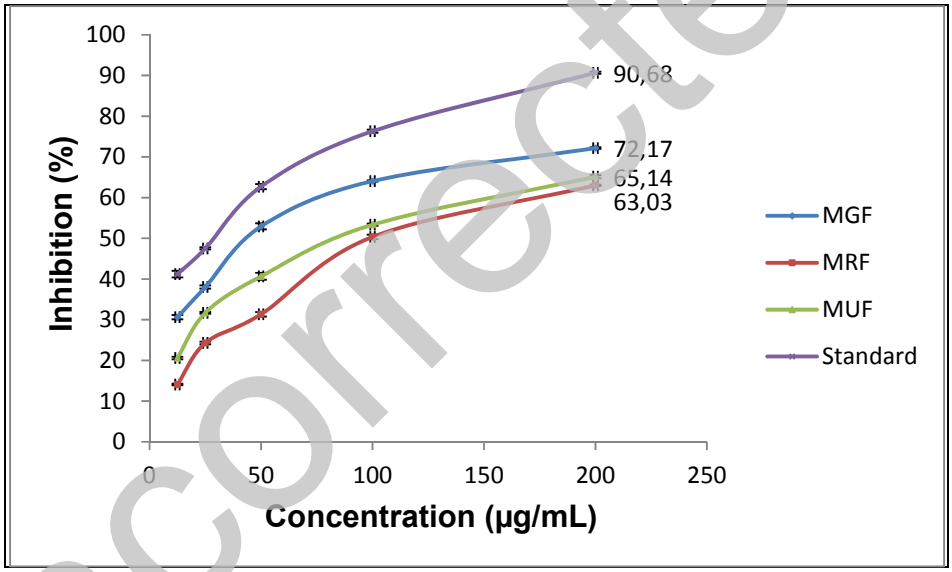



**Figure 11**: *In vitro* super oxide radical scavenging activity of *Memecylon* fruits




MGF: *Memecylon grande* fruits; MRF: *Memecylon randerianum* fruits; MUF: *Memecylon umbellatum* fruit.

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