



# Comparative Seed Morphology, Pharmacognostic, Phytochemical, and Antioxidant Potential of *Memecylon* L. Fruits

## *Memecylon* L. Meyvelerinin Karşılaştırmalı Tohum Morfolojileri, Farmakognostik, Fitokimyasal ve Antioksidan Potansiyelleri

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### ABSTRACT

**Objectives:** *Memecylon* is the genus of the family Melastomataceae and their identification is somewhat difficult as a result of similarities in their intraspecies morphologies. Thus, identification of these species is much strenuous. The present study investigated the seed morphology, pharmacognostic, phytochemical, and antioxidant potential of *Memecylon* fruits.

**Materials and Methods:** The species *Memecylon grande*, *Memecylon randerianum* and *Memecylon umbellatum* were selected for this study. The surface morphology of seeds or fruits, pharmacognostic evaluation, and phytochemical analysis are effective methods for rectifying taxonomic difficulties in authentication process and it opens a platform for pharmaceutical analysis. The surface morphology was analyzed by scanning electron microscopy (SEM) and elemental compositions were revealed by using energy dispersive X-ray spectroscopy (EDX) and inductively coupled plasma-mass spectroscopy (ICP-MS). While evaluating the pharmacognostic property of the seeds, powder microscopic analysis was used, which focused on the functional purity of the fruit samples. 2,2-diphenyl-1-picrylhydrazyl, hydroxyl, nitric oxide and superoxide radical scavenging assays are carried out to determine the antioxidant potential of *Memecylon* fruits.

**Results:** The surface morphology of seeds had a distinct pattern of topography. SEM and EDX studies gave an insight on the elemental composition of the seeds. ICP-MS analysis showed the presence of biologically potent trace elements in the fruit samples. Quantitative estimation of secondary metabolites, such as alkaloids, terpenoids, flavonoids, and phenols, revealed that fruit extracts possess a significant amount of these phytoconstituents. The present study also reveals the antioxidant potential of *Memecylon* fruit extracts.

**Conclusion:** Powder microscopy, SEM, EDX and ICP-MS analyses provided knowledge about the functional purity and elemental composition of *Memecylon* fruits samples. The free radical scavenging assays showed that *Memecylon* fruits were natural antioxidant sources. Therefore, these findings can be effectively targeted toward the pharmacological utilization of *Memecylon* fruits.

**Key words:** *Memecylon*, SEM, EDX, powder microscopy, secondary metabolites, antioxidants

### ÖZ

**Amaç:** *Memecylon*, Melastomataceae ailesine ait bir cinstir ve türler arası morfolojilerin belirlenmesinin zor olması nedeniyle ayırt edilmeleri zordur. Bu nedenle, bu türlerin ayırt edilmeleri güçtür. Bu çalışmada *Memecylon* meyvelerinin karşılaştırmalı tohum morfolojileri, farmakognostik, fitokimyasal ve antioksidan potansiyelleri incelenmiştir.

**Gereç ve Yöntemler:** *Memecylon grande*, *Memecylon randerianum* ve *Memecylon umbellatum* türleri bu çalışma için seçilmiştir. Tohumların veya meyvelerin yüzey morfolojileri, farmakognostik değerlendirmeleri ve fitokimyasal analizleri belgelendirme sürecinde taksonomik zorlukları rektifiye etmek için etkin yöntemlerdir. Yüzey morfolojileri taramalı elektron mikroskopisi (SEM) ile analiz edilmiştir ve elementel bileşimleri, enerji dağıtıcı X-ışını spektroskopisi (EDX) ve indüktif eşleşmiş plazma-kütle spektroskopisi (ICP-MS) ile açığa kavuşturulmuştur. Tohumların farmakognostik özelliklerini değerlendirirken, toz mikroskopik analizi kullanılmış ve meyve örneklerinin fonksiyonel saflığına odaklanılmıştır. 2,2-difenil-1-pikrilhidrazil, hidroksil, nitrik oksit ve süperoksit radikal süpürücü deneyleri *Memecylon* meyvelerinin antioksidan etkilerini belirlemek için gerçekleştirilmiştir.

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**Bulgular:** Tohumların yüzey morfolojileri belirgin topografik karakter göstermiştir. SEM ve EDX çalışmaları tohumların elementel bileşimi hakkında bilgi vermiştir. ICP-MS analizi meyve örneklerinde biyolojik olarak potent eser elementlerinin varlığını göstermiştir. Alkaloidler, terpenoidler, flavonoidler ve fenoller gibi sekonder metabolitlerin kantitatif olarak değerlendirilmesi meyve ekstraktlarının belirgin miktarda bu fitobileşenleri içerdiğini göstermiştir.

**Sonuç:** Toz mikroskopisi, SEM, EDX ve ICP-MS analizleri *Memecylon* meyve örneklerinin fonksiyonel saflık ve elementel bileşimleri hakkında bilgi vermiştir. Serbest radikal süpürücü yöntemleri *Memecylon* meyvelerinin doğal antioksidan kaynakları olduğunu göstermiştir. Bu nedenle, bu bulgular *Memecylon* meyvelerinin farmakolojik olarak kullanımlarının etkin olarak hedeflenmesini sağlayabilecektir.

**Anahtar kelimeler:** *Memecylon*, SEM, EDX, toz mikroskopisi, sekonder metabolitler, antioksidanlar

## INTRODUCTION

Green technology and alternative eco-friendly products are brand new to several.<sup>1</sup> New lifestyle changes cause many perilous drawbacks, which opens a gateway for the search of new resolves. Thus, at present, the term “Green” has become much popular. The major area under “Green” consideration will be the field of traditional or herbal medicine. Herbal medicines are safe remedies for various human ailments, given their minimal side effects and low-cost treatments. Thus, there is wide acceptance of herbal medicinal system. The quality measurements of herbs are challengeable streams, where validations of herbs are more important than their usage. Adulterations are drawbacks in the field of herbal medicine, as they cause inconsistencies in quality and safety of herbal preparations. This has opened a new approach to validate the quality assurance of herbs. The collection of plant materials, authentication of specimens, analysis, and formulation of drugs are the majorly value chain toward the discovery of safer natural drugs. Here in, an attempt was made to evaluate the 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 were reported and 21 among them are endemic to the country.<sup>2</sup> *Memecylon* species are difficult to identify due to the fact that their morphological similarities are some what confusing. *Memecylon umbellatum* and *Memecylon randerianum* are common species found in the Western Ghats. Another selected species for the present study is *Memecylon grande*, which is present in the Western Ghats and dry deciduous forests. Previous reports have revealed that these three species of *Memecylon* have potent medicinal activity.<sup>3,4</sup> *M. umbellatum* possesses elliptic-lanceolate leaves with umbellate inflorescence and yellow berries. *M. randerianum* possesses ovate-oblong leaves and blueberries, while *M. grande* has ovate-lanceolate leaves with brownish blackberries.

Many systematic studies and new records are available on the genus *Memecylon*; however, evaluations of micromorphological characters are trivial. Scanning electron microscopy (SEM) analysis is the best way to analyze the surface features of the samples. In taxonomic identification, analysis of vegetative and reproductive characters through SEM is an effective method.<sup>5</sup> The functional purity of plant samples is essential for pharmaceutical trails. In this present study, purity of sample was analyzed by powder microscopy, SEM-energy dispersive X-ray

(EDX), and inductively coupled plasma-mass spectrometry (ICP-MS) techniques. Powder microscopy acts as an effective tool for authentication plant materials.<sup>6</sup> SEM-EDX and ICP-MS are effective analytical methods for analyzing metal nano- and micro-particles in food products, plant, or environmental.<sup>7</sup> The backbone behind the performance of plants always hinge on the presence of bioactive metabolites. In majority of pharmaceutical studies, phytochemical analysis is a crucial step. Preliminary phytochemical analysis gives an insight on the phytochemical constituents present in plant extracts. It comprises qualitative and quantitative analysis. It provides valid information regarding the presence or absence of bioactive compounds in plant species. A plethora of biochemicals contribute to the specific bioactivity of plants, such as antimicrobial, antioxidant, and anticancer properties, among others.<sup>8</sup> The present study also emphasizes the evaluation of the antioxidant potential of *Memecylon* fruit extracts. Antioxidants are free radical scavengers that neutralize oxidative stress induced by reactive oxygen species (ROS). Furthermore, ROS can disrupt normal cellular mechanisms and lead to severe pathological conditions and diseases, such as cancer, neurological disorders, atherosclerosis, hypertension, ischemia, and diabetes.<sup>9</sup> The present study focused on the worth and utility of micromorphological characters and functional purity of fruit samples as an additional source of information in systematic studies of *Memecylon*.

## MATERIALS AND METHODS

### *Plant material*

Fruits of *M. grande*, *M. randerianum*, and *M. umbellatum* were chosen for the current study. Ripened mature fruits of the three species were collected from various parts of Kerala, India. The collected fruits were identified by Dr. A. K. Pradeep Assistant Professor, Angiosperm Taxonomy Division, Department of Botany, University of Calicut, Kerala.

### *SEM analysis*

SEM analysis was performed using ZEISS Gemini SEM 300 machine. 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:  $\mu$ 3 pA-20 Na. Magnification: 12-2,000,000.

### SEM-EDX analysis

SEM-EDX analysis was done using Octane plus with Gemini 300/EDS. The active area selected for the present study was 30 mm<sup>2</sup>.

### Powder microscopy

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

### ICP-MS analysis

ICP-MS analysis was performed using Agilent 7800 ICP-MS with Integrated Sample Introduction System (ISIS3) and SPS4 autosampler. It has a standard torch of 2.5 mm-diameter injector, as well as Ni sampler and Ni skimmer cones.

### Preliminary quantitative phytochemical analysis

#### Alkaloid content

The method of Shamsa et al.<sup>10</sup> was followed for the determination of total alkaloid content of the selected plant material. In brief, 2 N HCl solution was prepared and 1 mg of the plant sample was dissolved in it and filtered. Phosphate buffer (pH 4.7) was prepared and 5 mL of it and 5 mL BCG solution was added and the mixture was shivered with 1, 2, 3, and 4 mL of chloroform. The chloroform layer containing alkaloids was separated. Caffeine was used as a standard. Absorbance of the solution was read at 470 nm against a blank. Alkaloid content of the sample was calculated and expressed as mg of caffeine equivalents.

#### Flavonoid content

Aluminium chloride colorimetric assay is a typical method for the determination of flavonoid content.<sup>11</sup> 4 mL of distilled water was put in a flask and 1 mL of the extracts was added into it. 0.30 mL of 5% NaNO<sub>2</sub> and 0.3 mL of 10% AlCl<sub>3</sub> were mixed in the flask at five minutes intervals. Again, after five minutes incubation, 2 mL of 1M NaOH was taken and the final volume of 10 mL was attained using distilled water. Finally, absorbance was read at 510 nm. The total flavonoid present in the extract was calculated and expressed as mg quercetin equivalents (QE).

#### Phenolic content

Phenolic content of the sample was assayed by Folin Ciocalteu assay.<sup>12</sup> 1 mL of the extracts was put in a 25 mL flask containing 9 mL of distilled water. 1 mL of Folin-Ciocalteu phenol reagent was mixed in the flask and shaken. At 5 minutes interval, 10 mL of 7% Na<sub>2</sub>CO<sub>3</sub> solution was added and the solution was kept for 90 minutes at room temperature. Absorbance was read at 550 nm against the reagent blank. Gallic acid was taken as the standard. The phenolic content was expressed as mg of Gallic acid equivalents.

#### Terpenoid content

Presence of terpenoid of the plant samples was estimated by the method of Ghorai et al.<sup>13</sup> The reaction solution contains an aliquot of the extract and few drops of chloroform and H<sub>2</sub>SO<sub>4</sub>.

Absorbance is calculated at 538 nm against the blank. Linalool was taken as the standard. The terpenoid content of the extract was calculated and expressed as mg linalool equivalents (QE).

### Antioxidant activity

The antioxidant potential of *Memecylon* fruits was documented on the basis of free radicals produced by various substrates, such as 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH), Fe<sup>3+</sup>-ascorbate-EDTA-H<sub>2</sub>O<sub>2</sub> system, sodium nitroprusside, and potassium ferricyanide.

#### DPPH radical scavenging assay

The protocol of Chang et al.<sup>14</sup> was followed for the determination of DPPH free radical scavenging activity of *Memecylon* fruit extract. DPPH is a free radical that reacts with antioxidant agents and gets reduced to DPPH-H. The pink colored DPPH turns yellow when scavenged by antioxidants. The color change indicates the scavenging latent of the antioxidant substances. Reference was selected as ascorbic acid [10 mg/mL dimethyl sulfoxide (DMSO)]. Different volumes of the extracts [1.25-20 µL (12.5-200 µg/mL)] from a stock concentration of 10 mg/mL was taken and made up to 20 µL with DMSO and 1.48 mL of DPPH (0.1 mM) solution. The control was a test compound-free solution. A dark condition of 20 minutes was maintained for the reaction mixture at room temperature. Then, the scavenging potential was read at 517 nm. Percentage inhibition of DPPH free radical by the extracts was calculated as follows:

$$\% \text{ inhibition} = \frac{\text{control} - \text{test}}{\text{control}} \times 100$$

#### Hydroxyl radical scavenging activity

Hydroxyl radical scavenging potential was analyzed according to the method described by Kunchandy and Rao.<sup>15</sup> Different concentration of the samples (125-2000 µg/mL) from a stock solution of 10 mg/mL was mixed with 500 µL reaction mixture [2-deoxy 2-ribose (2.8 mM), FeCl<sub>3</sub> (100 µM), EDTA (100 µM), H<sub>2</sub>O<sub>2</sub> (1.0 mM), ascorbic acid (100 µM) in KH<sub>2</sub>PO<sub>4</sub>-KOH buffer (20 mM pH 7.4)] and made up to 1 mL. The control was a solution devoid of the test material. The test sample tubes were kept at 37°C for 1 hour. 1 mL of 2.8% TCA and 1 mL of 1% aqueous TBA was mixed in the reaction tubes and allowed to stay for 15 minutes at 90°C for the color change. Absorbance was read at 532 nm against a blank. Gallic acid (10 mg/mL DMSO) was used as reference. Percentage inhibition of hydroxyl radical by the extracts was calculated as follows:

$$\% \text{ inhibition} = \frac{\text{control} - \text{test}}{\text{control}} \times 100$$

#### Nitric oxide radical scavenging activity

Determination of nitric oxide radical scavenging activity of *Memecylon* fruit extracts was performed according to the method described by Kumaran and Karunakaran.<sup>16</sup> Sodium nitroprusside (5 m/mole/L<sup>-1</sup>) in phosphate buffered saline solution (pH 7.4) was added into various concentrations of extracts (125-2000 µg/mL) from a stock solution. The reaction mixtures were then

incubated at 25°C for 30 minutes. A control was also prepared and incubated accordingly. After 30 minutes incubation, 1.5 mL of the reaction mixtures were removed and 1.5 mL of Griess reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% N-1-naphthylethylenediamine dihydrochloride) was added. Absorbance was read at 546 nm. Gallic acid (10 mg/mL DMSO) was used as a reference compound. Percentage inhibition of nitric oxide radical by the extracts was calculated as follows:

$$\% \text{ inhibition} = \frac{\text{control-test}}{\text{control}} \times 100$$

#### Superoxide radical scavenging activity

Superoxide radical scavenging assay was performed according to the method described by Valentão et al.<sup>17</sup> A concentration range of (125-2000 µg/mL) was taken from a stock solution (10 mg/mL). The reaction tube contained 0.05 mL of riboflavin solution (0.12 mM), 0.2 mL of EDTA solution (0.1 M), and 0.1 mL of nitro blue tetrazolium solution (1.5 mM). 2.64 mL of phosphate buffer (0.067 M) was added to the reaction mixture. Absorbance was read at 560 nm at two illumination times of 5 minutes and 30 minutes, respectively. Ascorbic acid (10 mg/mL DMSO) was used as reference. Percentage inhibition of superoxide radical by the extracts was calculated as follows:

$$\% \text{ inhibition} = \frac{\text{control-test}}{\text{control}} \times 100$$

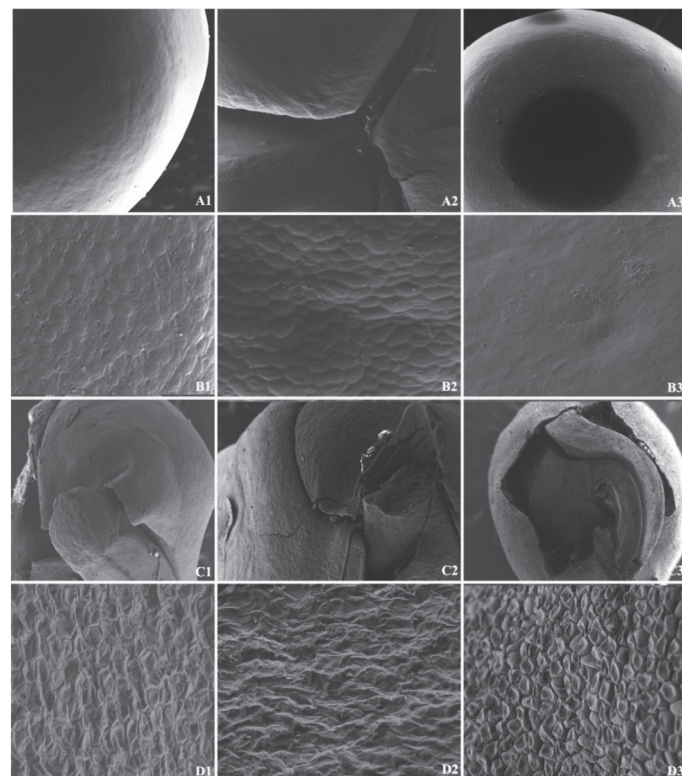
## RESULTS AND DISCUSSION

SEM is a high-resolution surface imaging technique that uses an electron beam with superior magnification power and depth of field precision. The seed capsule micromorphology and the entire seed morphology were studied by SEM analysis. The difference in electron emission in different areas provides the surface topography of the material. In this study, all the selected species showed distinct morphological patterns. Surface morphology is an important index in taxonomic identification.<sup>18</sup> SEM analysis of *Memecylon* fruits is a novel report. In the case of *M. grande*, fruits show a colliculate pattern, with a seed capsule of 6.2 mm, and the seed surface possesses a tuberculate pattern, with a width of 5.8 mm (Figure 1 A1, B1, C1, and D1). SEM analysis revealed that the seed capsule of *M. randerianum* a ruminate reticulate type pattern (Figure 1 A2, B2, C2, and D2). The width of the capsule was 4.2 mm. The seed surface of *M. randerianum* was of a reticulate pattern, with a width of 3.5 mm. *M. umbellatum* seed capsule possesses a smoothened pattern, with a width of 5.6 mm, and its seed surface showed a wrinkled pattern, with a width of 3.81 mm (Figure 1 A3, B3, C3, and D3). The characteristic surface morphology is a useful tool in the identification process.

EDX is an effective way of analyzing elemental compositions at tiny level. In this study, SEM was coupled with an energy dispersive system with an electron probe for X-ray microanalysis. The surfaces of the specimens were evaluated

by SEM-EDX analysis and this gave a clear picture of the elemental composition of the test sample.<sup>19</sup> The elemental composition of *M. grande* fruits show that nitrogen content was 91%, while that of other elements were as follows: 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 was the prominent element, with 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%) were the composition of other elements (Figure 3). *M. umbellatum* fruit also possesses an elevated amount of nitrogen (93%) and the other elements were 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 indicates that *Memecylon* fruits are reservoirs of essential elements and can be exploited in the pharmaceutical or nutritional field.

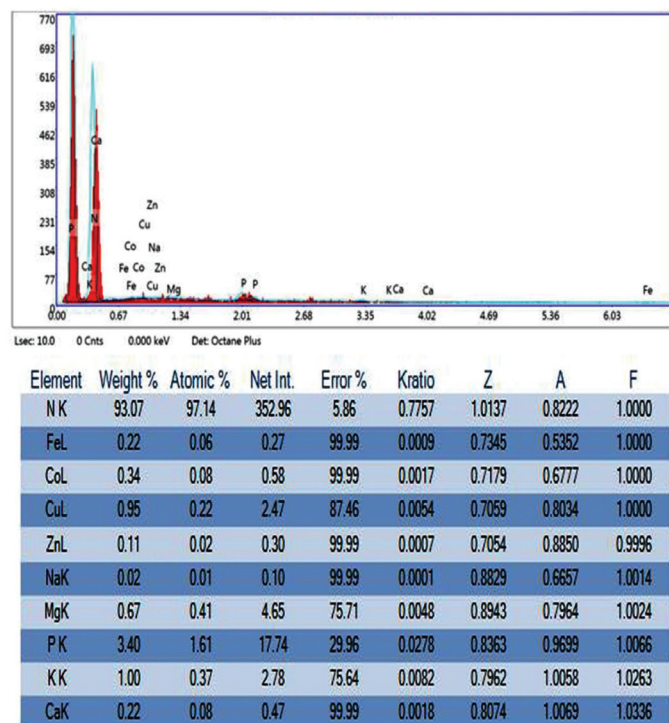
In Ayurveda, 90% of the preparations are plant-based, implying that the worthwhile usage of herbal medicines are promising remedies for diverse human diseases. In most Ayurvedic preparations, the powdered samples of plant parts are used. Therefore, the authenticity of the powdered sample is very important. Powder microscopy is a simple and easier method to analyze powdered samples and is an essential step in pharmacognostic evaluation of plant samples. Microscopic techniques examine the structural and cellular features of herbs, so as to determine their botanical origin. Microscopic



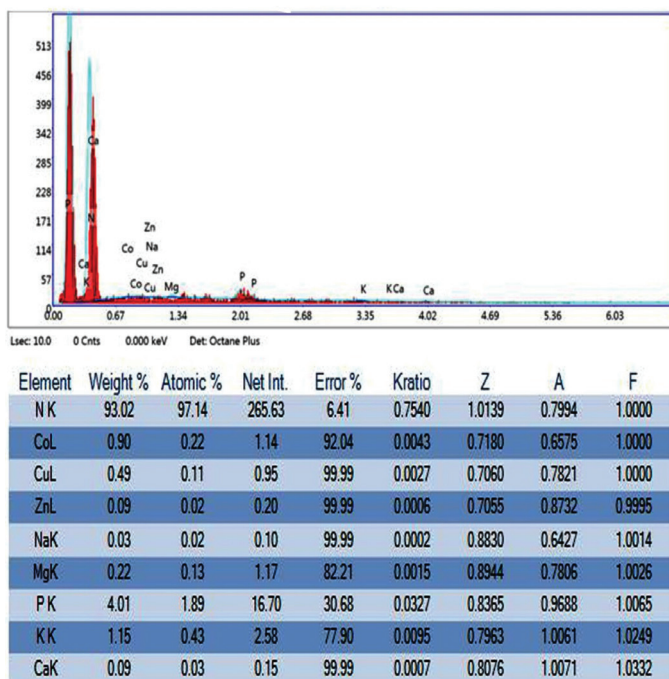
**Figure 1.** Scanning electron microscopic analysis of *Memecylon* fruits. A1) *Memecylon grande* seed capsule, B1) enlarged view, C1) seed surface, D1) Enlarged view. A2) *Memecylon randerianum* seed capsule, B2) enlarged view, C2) seed surface, D2) enlarged view. A3) *Memecylon umbellatum* seed capsule,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



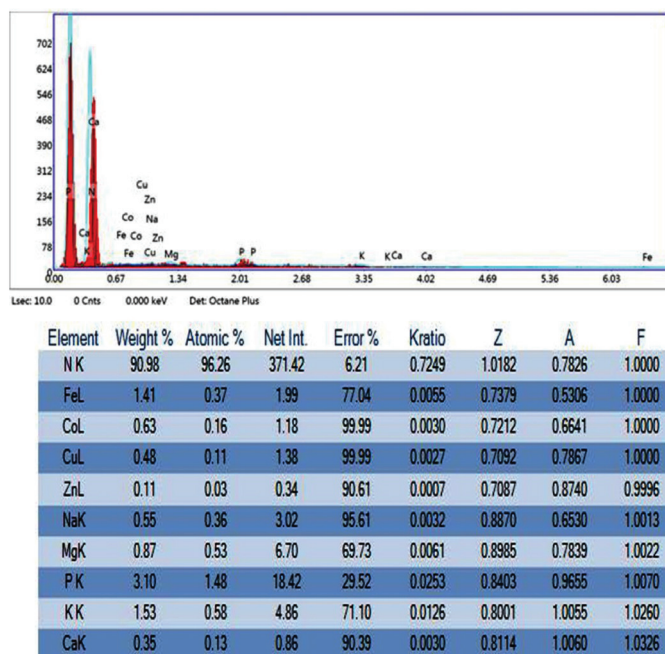
evaluation is now an indispensable tool for the identification of medicinal herbs and is among the important parameters in this modern time.<sup>20</sup> Here in, the powdered samples of *Memecylon* fruits were characterized by their microscopic characters. The powdered *M. grande* fruits was brown in color, odorless, and slightly astringent (Figure 5). The characters found in the



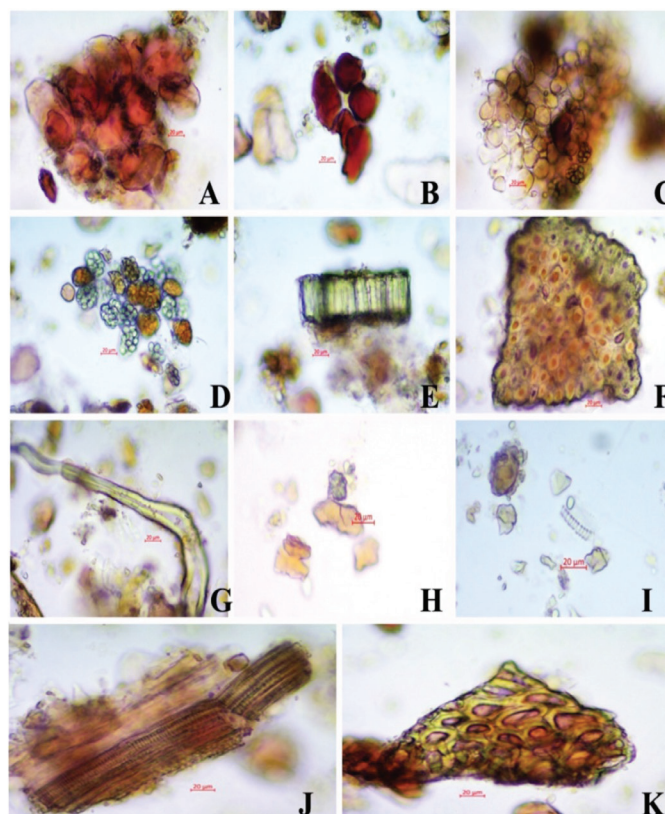
**Figure 2.** SEM-EDX analysis of *Memecylon grande* fruits  
SEM: Scanning electron microscopy, EDX: Energy dispersive X-ray



**Figure 3.** SEM-EDX analysis of *Memecylon randerianum* fruits  
SEM: Scanning electron microscopy, EDX: Energy dispersive X-ray



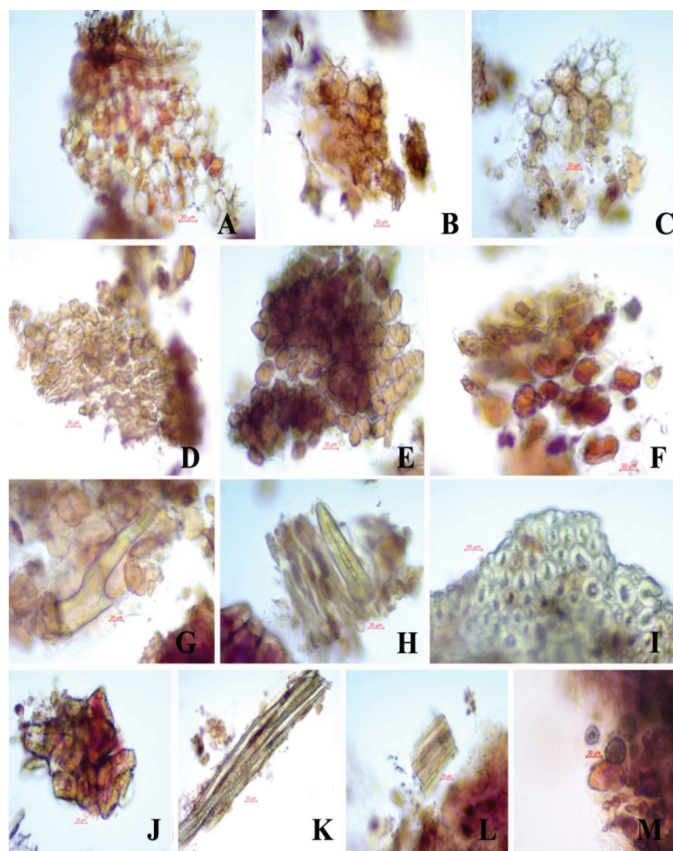
**Figure 4.** SEM-EDX analysis of *Memecylon umbellatum* fruits  
SEM: Scanning electron microscopy, EDX: Energy dispersive X-ray



**Figure 5.** Powder microscopic analysis of *Memecylon 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



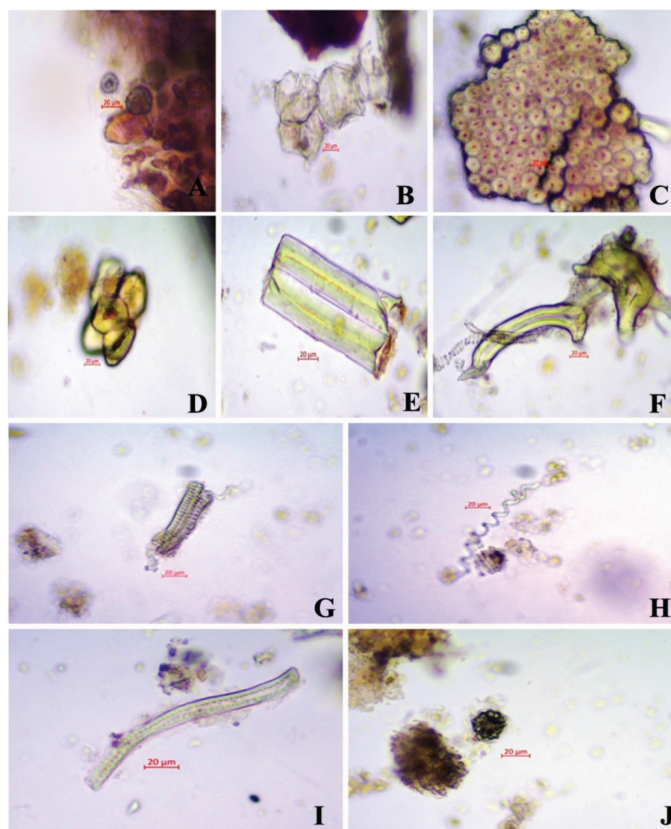
powders were epicarp cells, parenchyma cells with starch grains from mesocarp, stone cells from mesocarp, sclereids from endocarp, vessels with spiral and annular thickenings, and rosette crystals. In the case of *M. randerianum* fruits powder, it is brown-colored, odorless with a characteristic taste (Figure 6), and contains epicarp cells, mesocarp parenchyma cells, stone cells, sclereids from endocarp, tracheids, fiber bundles, and rosette crystals. The same brown colored powder was also seen for *M. umbellatum* fruits (Figure 7), which showed characteristics, such as epicarp cells, pitted parenchyma cells from mesocarp, stone cells, sclereids, spiral vessels, fibro-sclereids, and rosette crystals. These characters can be used to identify authenticated plant specimens in Ayurvedic preparations. Therefore, we can easily identify the botanical origin of the plant specimen and clearly distinguish the presence of adulterants or allied species. Microscopic evaluation of *M. umbellatum* leaves was done according to the method described by Killedar et al.<sup>6</sup> and the presence of lignified xylem with well-defined xylem fibers, vessels, and parenchyma was found. The presence of phloecentric vascular bundles surrounded by endodermis and crystal sheath was also reported. The powder microscopic analysis confirmed that the botanical origins of the plant samples were pure and devoid of foreign particles. Therefore, this result can be used as a future reference for identification of *Memecylon* fruits.



**Figure 6.** Powder microscopic analysis of *Memecylon randerianum* fruits. A) Mesocarp in sectional view; B) epicarp in surface view; C-F) mesocarp cells; G, H) sclereids; I, J) stone cells; K) tracheids; L) fiber bundles; M) rosette crystals

During the past decades, much attention have been focused on nutritional status. We had an insight into the profound effect of micro and macronutrients on biological processes that range from whole-organism performance to cellular function. According to the classification of trace elements, group I, which consists of carbon, hydrogen, oxygen, and nitrogen, are the key building blocks of carbohydrates, proteins, and lipids. Group II includes nutritionally important minerals, such as sodium, potassium, phosphorous, chloride, calcium, magnesium, and sulfur. Trace elements, such as like copper, iron, zinc, chromium, cobalt, iodine, molybdenum, and selenium, are found in group III.<sup>21</sup> Copper is important in metabolism, mainly in the proper functioning of the enzymes and its deficiency can lead to hypochromic anemia, joint pain neutropenia, hypopigmentation of hair and skin, abnormal bone formation with skeletal fragility, and osteoporosis, among others.<sup>22</sup> 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 vital cause of maternal mortality in India and 20% of the mortality is directly related to anemia, while another 50% is associated with other anemic side effects. In the case of zinc, it is crucial for normal spermatogenesis and maturation, development of thymus, epithelialization in wound healing, taste sensation, and secretion of pancreas and gastric enzymes.<sup>23</sup>

In addition to the SEM-EDX analysis, to substantiate the quality of the fruit samples in their elemental diversity, ICP-



**Figure 7.** Powder microscopic analysis of *Memecylon 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

MS analysis was performed. This technique gave details of the elements present in the sample in parts per million and determination of thirteen elements, including aluminum, arsenic, cadmium, cobalt, strontium, selenium, chromium, copper, molybdenum, nickel, lead, barium, and manganese, were done. *M. grande* fruits show a promising concentration of majority of the 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 adult human blood samples are considered, considering that it is essential for the standardization of drugs. Most of the detected elements show vital biological functions. Some elements are functional parts of vitamins, cofactors of enzymes, oxidative phosphorylation, fatty acids, and cholesterol metabolism. Lead is considered as a non-essential elemental part of the human body, since it has no biological or health roles. In the case of chromium, it acts as a modulator of enzymes and as an activator of fatty acid and cholesterol metabolism. It can also enhance sugar metabolism.<sup>24</sup> In the case of cobalt, it is the key factor of cobalamin (vitamin B12) and plays a vital role in the production of amino acids and neurotransmitters. Although the biological function of nickel is still somewhat unclear, nickel is found in higher concentration in RNA and is involved in protein structure.<sup>25</sup> Therefore, the biological role of these trace elements is significant in the

maintenance homeostasis and avoidance of free radical proliferation induction and various human ailments.<sup>26</sup>

Preliminary quantitative analysis of various phytochemicals was done following the proposed 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<sup>27</sup> 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,<sup>28</sup> which includes antifungal, antihyperglycemic, antityrosinase, antiglycosidase, antinociceptive, and antiinflammatory activities, among others. The efficiency of bioactive products in curing several diseases, such as hyperlipidemia, atherosclerosis, and hypertension, were studied by Liwa et al.<sup>29</sup> Polyphenols and phenolic compounds are full sources of vascular vasodilators and are used to cure hypertension and cardiovascular diseases. Flavonoids possess anti-inflammatory, anticancerous, and antimutagenic activities, which have been previously reported.<sup>30</sup> Tan et al.<sup>31</sup> confirms that the progressive use of phytochemicals through diets is an effective method to cure diseases. *Memecylon* fruit extract showed a promising antioxidant activity in four different assays. All the fruit extracts show a dose gradient scavenging activity.

**Table 1. ICP-MS analysis of *Memecylon* fruits**

Element	Mass	Concentration (ppm)			Reference concentration of trace elements in adult human blood (µg/L) <sup>20</sup>
		MGF	MRF	MUF	
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

1 µg/L: 0.001 ppm. ICP-MS: Inductively coupled plasma-mass spectroscopy, MGF: *Memecylon grande* fruit, MRF: *Memecylon randerianum* fruit, MUF: *Memecylon umbellatum* fruit

Among the extracts, *M. grande* fruit extract showed the highest free radical scavenging activity in all the assays, followed by *M. umbellatum* and *M. randerianum* fruit extracts (Figure 8-11). *M. grande* fruit extracts showed that highest nitric oxide scavenging activity ( $76.85 \pm 0.08$ ) and the least hydroxyl radical scavenging activity ( $61.69 \pm 0.56$ ). *M. randerianum* fruit showed the least hydroxyl radical scavenging activity ( $46.16 \pm 0.17$ ). Based on the four assays performed, the most effective antioxidant extracts was that of *M. grande* fruit, with an  $IC_{50}$  of  $83.9195 \pm 0.14$ . This was followed by *M. umbellatum*, with an  $IC_{50}$  of  $91.1031 \pm 0.12$ , and *M. randerianum*, with an  $IC_{50}$  of  $104.178 \pm 0.13$  (Table 3). Antioxidant capacity and total phenolic content are directly related.<sup>32</sup> The quantitative phytochemical analysis justifies the high performance of these species in the antioxidant assays. *M. grande* fruit extract ( $370.28 \pm 8.36$ ) possessed the highest

phenolic content, making it a good candidate pharmaceutical agent.

#### Study limitations

The detailed phytochemical characterization and compound isolation can be more satisfactory in pharmaceutical applications. The present findings are useful in the pharmaceutical field, since the botanical purification of plants is the prime step in natural drug formulation. These results can be used as a future reference for the evaluation of *Memecylon* fruits.

#### CONCLUSION

Seed surface characteristics are used as diagnostic tools in taxonomic studies. Powder microscopy, SEM-EDX, and ICP-MS analysis have provided knowledge about the functional purity and elemental composition of *Memecylon* fruits samples. The

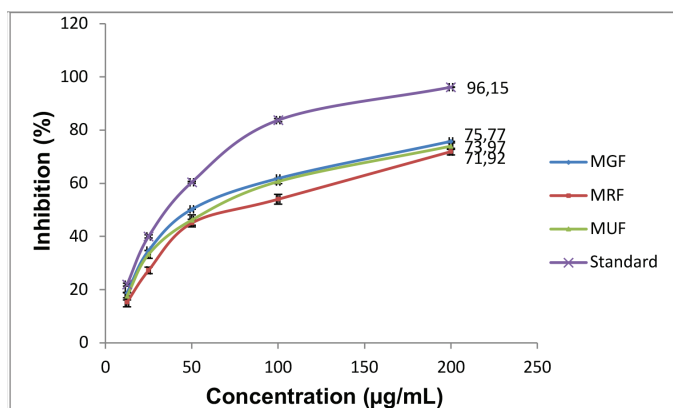


Figure 8. *In vitro* DPPH scavenging activity of *Memecylon* fruits

DPPH: 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl, MGF: *Memecylon grande* fruit, MRF: *Memecylon randerianum* fruit, MUF: *Memecylon umbellatum* fruit

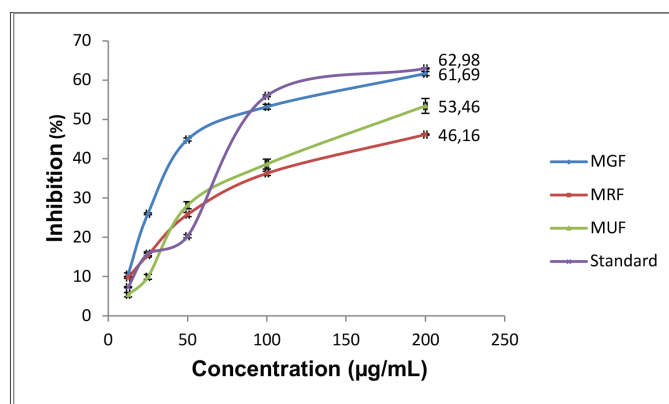


Figure 9. *In vitro* hydroxyl radical scavenging activity of *Memecylon* fruits

MGF: *Memecylon grande* fruit, MRF: *Memecylon randerianum* fruit, MUF: *Memecylon umbellatum* fruit

Table 2. Preliminary quantitative phytochemical analysis of *Memecylon* fruits

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

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.

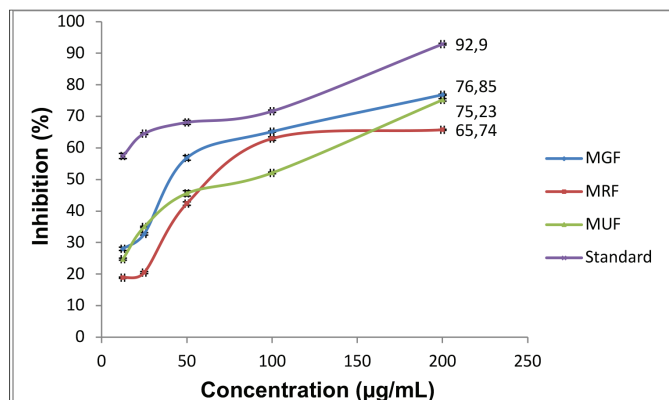
SE: Standard error, DW: Dry weight, GAE: Gallic acid equivalent, MGF: *Memecylon grande* fruit, MRF: *Memecylon randerianum* fruit, MUF: *Memecylon umbellatum* fruit

Table 3. Effect of methanol extracts of *Memecylon* fruits in different antioxidant assays

$IC_{50}$ values ( $\mu$ g/mL)				
Plants	DPPH radical scavenging assay	Hydroxyl radical scavenging assay	Nitric oxide radical scavenging assay	Super oxide radical scavenging assay
Standard	$48.8412 \pm 1.5^a$	$1347.51 \pm 0.27^b$	$346.207 \pm 0.01^a$	$238.357 \pm 0.03^a$
MGF	$83.9195 \pm 0.14^b$	$1231 \pm 0.48^a$	$696.733 \pm 0.06^b$	$698.991 \pm 0.03^b$
MRF	$104.178 \pm 0.13^d$	$2029.57 \pm 0.14^e$	$1081.61 \pm 0.01^d$	$311.24 \pm 0.02^e$
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. DPPH: 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl, Correct it as MGF: *Memecylon grande* fruit, MRF: *Memecylon randerianum* fruit, MUF: *Memecylon umbellatum* fruit





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

MGF: *Memecylon grande* fruit, MRF: *Memecylon randerianum* fruit, MUF: *Memecylon umbellatum* fruit

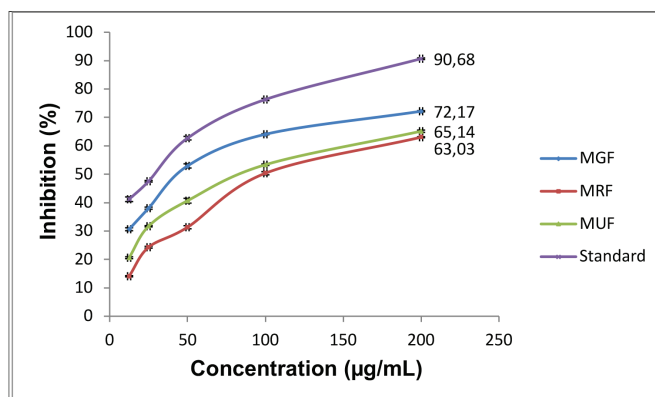
free radical scavenging assays provide an acquaintance of a natural antioxidant source. Therefore, these findings can be effectively targeted toward the pharmacological utilization of *Memecylon* fruits.

The present investigation focuses on the seed morphology, pharmacognostic, phytochemical, and antioxidant potential of *Memecylon* fruits. *M. grande*, *M. randerianum* and *M. umbellatum* are the selected experimental plant materials. Powder microscopy, SEM-EDX and ICP-MS analysis were carried out to analyze the surface features and elemental composition of the *Memecylon* fruits. The antioxidant potential of fruit samples are analyzed by DPPH, hydroxyl, super oxide and nitric oxide radical scavenging assays. The analysis of morphological features are key diagnostic tools in taxonomic studies. In the present study SEM analysis of fruit samples gave valuable results in species identification of *Memecylon*. The each sample shows distinct surface morphology. In ICP-MS analysis, elemental composition of fruit samples reveals the diverse elemental presence in the sample. It may helpful in pharmaceutical field for valuable medicine preparations. In the four antioxidant assays, all the selected extracts shows promising free radical scavenging activity. So all these results pointing that selected *Memecylon* fruit samples become a potential contributor in the pharmaceutical field.

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

MGF: *Memecylon grande* fruit, MRF: *Memecylon randerianum* fruit, MUF: *Memecylon umbellatum* fruit

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