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PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT POTENTIAL OF *FICUS BENGHALENSIS* L.

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ABSTRACT

Genus *Ficus* belongs to family Moraceae having 40 genera and over 1000 species worldwide. Different methods have been used for phytochemical screening of medicinal plants like total phenolics content (TPC) and total flavonoids content (TFC) assays to quantify phenolics and flavonoids. The phytochemical analysis exhibited highest total phenolics content in M extract of stem and total flavonoids content in ethyl acetate (EA) extract of leaves i.e. 61.2±1.3 µg GAE/mg extract and 25.1±0.9 µg QE/mg extract respectively. Total reduction power and total antioxidant capacity were maximum in the M extract of stem i.e. 243.89±1.6 µg AAE/mg extract and 127.08±2.7 µg AAE/mg extract respectively.

Keywords: Phytochemical, Antioxidant, Medicinal plants.

INTRODUCTION

Genus *Ficus* belongs to family Moraceae having 40 genera and over 1000 species worldwide. It is also known as fig which plays an important role in ecosystem by providing fruit throughout the year to the insects, birds and animals (Chaudhary *et al*., 2012). *F. benghalensis* leaves contain leucopelargonin, bengalenoside, rutin, β amyrin along with psoralen, β sisterol, bergapten, quercetin-3-galactoside20, leucodelphinidin derivative, A glucoside and leucocynidin derivatives (Joseph and Raj, 2010b).

Phytochemicals are chemically diversified group of compounds produced by plant as a part of defense against pathogens, predators and competitors. They have specific chemical properties that are useful for humans in treatment of diseases such as antioxidant, antimicrobial, interference with DNA replication and inhibition or stimulation of enzymes (Halliwell, 2007). Phytochemicals responsible for broad spectrum of bioactivities include alkaloids, glycosides, tannins, polyphenols, flavonoids and many more (Joseph and Raj, 2010a). Flavonoids are bioactive leads with the ability to chelate and reduce metal ions (Nazli *et al.*, 2018). Different methods have been used for phytochemical screening of medicinal plants like total phenolics content (TPC) and total flavonoids content (TFC) assays to quantify phenolics and flavonoids (Angelova *et al.*, 2008).

Antioxidants in optimal range inhibit the process of oxidation to retain normal physiology of body. Plants possess numerous antioxidants which play an essential role as radical scavengers and transform free radicals into less reactive species. (Shahwar *et al.*, 2012). Antioxidant

potential is determined by a set of different assays because one technique is not sufficient to predict exact antioxidant potential. These techniques involve different mechanisms e.g. prevention of chain initiation, scavenging of radicals and disintegration of peroxides (Aliyu *et al.*, 2012).

MATERIALS AND METHODS

Phytochemical analysis

a) Assessment of TPC

A well stated protocol recounted by Fatima *et al.* (2015) was adopted for the estimation of total phenolics content in test extracts. The results were expressed as µg gallic acid equivalent (GAE)/mg extract of the plant.

b) Assessment of TFC

Procedure

A well stated procedure described by Khan *et al.* (2015) was performed for the estimation of total flavonoids content in test extracts (4 mg/ml DMSO). The results were expressed in µg quercetin equivalent (QE)/mg extract of plant.

Antioxidant assays

a) DPPH assay

Procedure

A well-defined procedure described by Ahmed *et al*. (2017) was used. Samples that showed a scavenging potential of more than 50% at initial concentrations were further analyzed for their IC_{50} values using three-fold serial dilution methodology.

b) Total antioxidant capacity (TAC)

Procedure

A well stated procedure described by Fatima *et al*. (2015) was adopted for the calculation of TAC of test extracts. The results were stated as µg ascorbic acid equivalent (AAE)/mg extract.

c) Total reducing power (TRP)

Procedure

A well-defined procedure elaborated by Zahra *et al*. (2017) was followed for the estimation.

RESULTS

Phytochemical Analysis

a) Total phenolics content

TPC was determined from all extracts of *F. benghalensis* and the results were expressed as µg gallic acid equivalent per mg extract (µg GAE/mg extract). Highest quantities of phenolics were quantified in M of stem, bark, leaves, adventitious root, fruit and root with values i.e. 61.19±1.3, 59.51±1.7, 59.42±1.0, 56.99±0.4, 55.61±0.5 and 46.07±0.7 µg GAE/mg extract respectively. Lowest phenolics content were calculated in NH extract of stem, bark, root, adventitious root, leaves and fruit with values i.e. 7.34±0.9, 6.53±1.4, 6.30±0.5, 5.62±0.4, 4.45±1.0 and 3.82±0.9 µg GAE/mg extract respectively. TPC in different test samples of *F. benghalensis* decreased in the following order: M>EA>DW>NH (Figure 1).

b) Total flavonoids content

TFC was determined from all extracts of *F. benghalensis* and results were expressed as µg quercetin equivalent per mg extract (µg QE/mg extract). Maximum TFC was given by EA extract of leaves part with value of 25.08±09 µg QE/mg extract followed by M (12.68±0.2 µg QE/mg), DW (9.11±1.5 µg QE/mg) and NH (5.26 µg QE/mg) extracts respectively. Following decreasing trends were observed in bark, stem, adventitious root and fruit i.e. M>DW>NH>EA, M>DW>EA>NH,DW>M>EA>NH,M>EA>

NH>DW respectively. Root extracts depicted minimum values i.e. M (7.14±0.3 µg QE/mg) followed by DW (5.96±1.7 µg QE/mg), EA (5.89±0.3 µg QE/mg) and (NH 4.91±0.1 µg QE/mg) extracts respectively (Figure 1).

Antioxidant assays

a) DPPH assay

A Free radical scavenging assay was performed and change in color from purple to yellow was measured. Maximum reducing activity in fruit was shown by M extract with an IC_{50} value of 3.18 µg/ml. In case of adventitious root, highest potential was observed in DW extract with an IC_{50} value of 4.10 µg/ml, while the M extract of leaves showed maximum potential with an IC_{50} value of 10.02 µg/ml. Highest activity in bark was shown by M extract with an IC_{50} value of 13.41 µg/ml, while DW extract of stem showed maximum potential with an IC_{50} value of 16.67 µg/ml. In case of root, M extract showed highest activity with an IC_{50} value of 20.98 µg/ml. The lowest radical scavenging activity was calculated in NH extract of adventitious root, bark, root, leaves, stem and fruit with % scavenging of 5.61, 4.21, 1.67, 0.79, 0.01 and 0.00 % respectively. The radical scavenging potential in different samples of *F. benghalensis* decreased as follows: M>DW>EA>NH in leaves, M>EA>DW>NH in fruit, M>DW>EA>NH in stem, M>DW>EA>NH in adventitious root, M>DW>EA>NH in bark and M>DW>EA>NH in root (Fig 2 & 3).

b) Total antioxidant capacity (TAC)

TAC of all the extracts of *F. benghalensis* was determined by Phosphomolybdenum based antioxidant assay and results are expressed as µg equivalent of ascorbic acid per mg extract (µg AAE/mg extract). The maximum TAC was observed in M extract of stem, leaves, bark, adventitious root, fruit and root with values i.e. 127.08±3.7, 106.76±0.4, 106.76±0.34, 90.74±1.4, 70.93±2.4 and 61.31±0.9 µg AAE/mg extracts respectively. Lowest antioxidant capacity was calculated in DW extract of leaves, fruit, stem and root with values i.e. 11.56±1.6, 6.08±3.8, 6.33±3.2 and 6.67±1.7 µg AAE/mg extract respectively, while NH extract of adventitious root and bark also showed comparatively low antioxidant potential with values i.e. 24.00±3.6 and 9.25±1.0 µg AAE/mg extract respectively. TAC of all samples displayed the following trend: M>EA>NH>DW in stem, M>EA>DW>NH in bark, M>EA>NH>DW in leaves, M>EA>DW>NH in adventitious root, M>EA>NH>DW in fruit and EA>M>NH>DW in root respectively (Fig 4 & 6).

c) Total reducing power

F. benghalensis extracts were analyzed for total reducing power and results are expressed as µg AAE/mg extract. Maximum TRP was quantified in M extract of stem, leaves, adventitious root, fruit, bark and root with values of 243.9±1.6, 244.9±2.6, 178.1±3.1, 158.3±2.5, 153.8±2.4 and 135.1±3.1 µg AAE/mg extract respectively. Lowest TRP values were observed in NH extract of adventitious root, root, stem, fruit and bark having value i.e. 49.68±1.3, 42.24±1.6, 40.06±2.0, 28.22±2.2 and 29.33±0.9 µg AAE/mg extract respectively.

TRP of all extracts showed decreasing trend as: M>EA>NH>DW in leaves, M>EA>DW>NH in stem, M>DW>EA>NH in adventitious root, M>EA>DW>NH in

root, M>EA>DW>NH in fruit and M>DW>EA>NH in bark (Fig 5 & 6).

DISCUSSION

Phytochemical Analysis

Medicinal plants naturally synthesize aromatic secondary metabolites which are responsible for pharmacological response in humans. Therapeutically active subcategories include phenolics, tannins, alkaloids, flavonoids etc. (Angelova *et al.*, 2008).

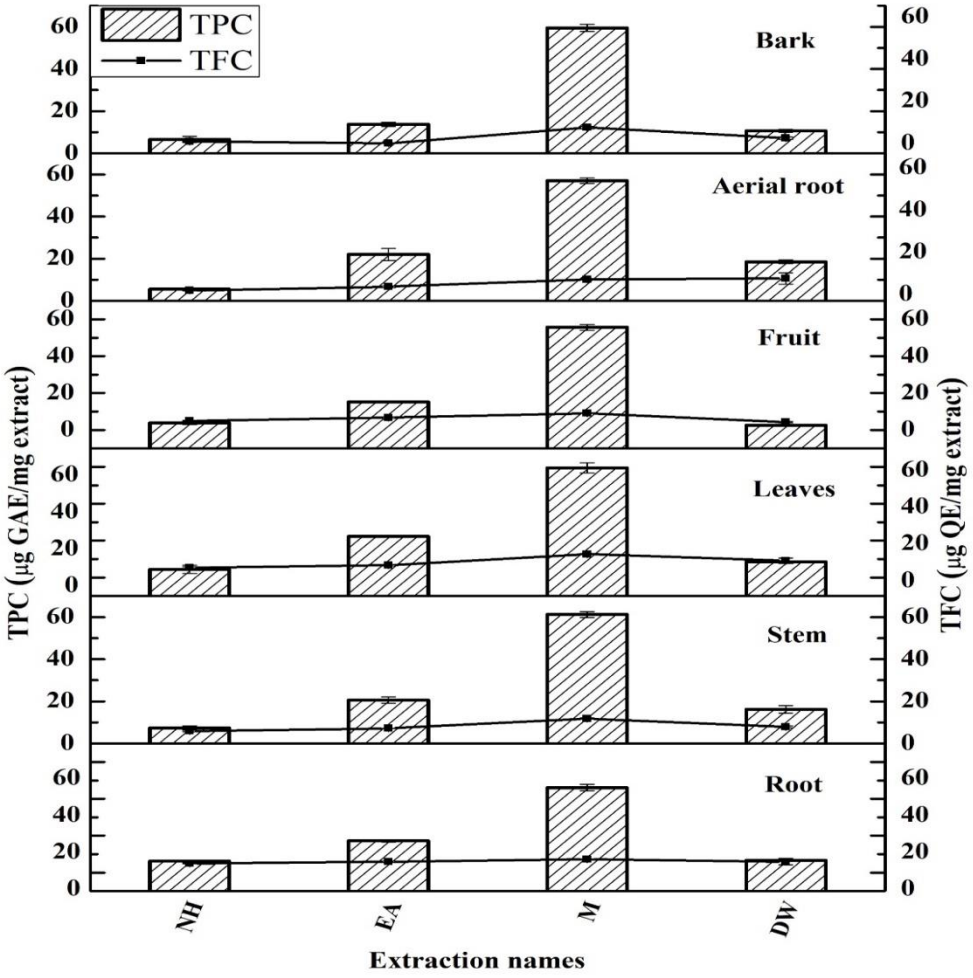

Figure 1. TPC and TFC of samples. Values presented are expressed as a mean of triplicate ± standard deviation.

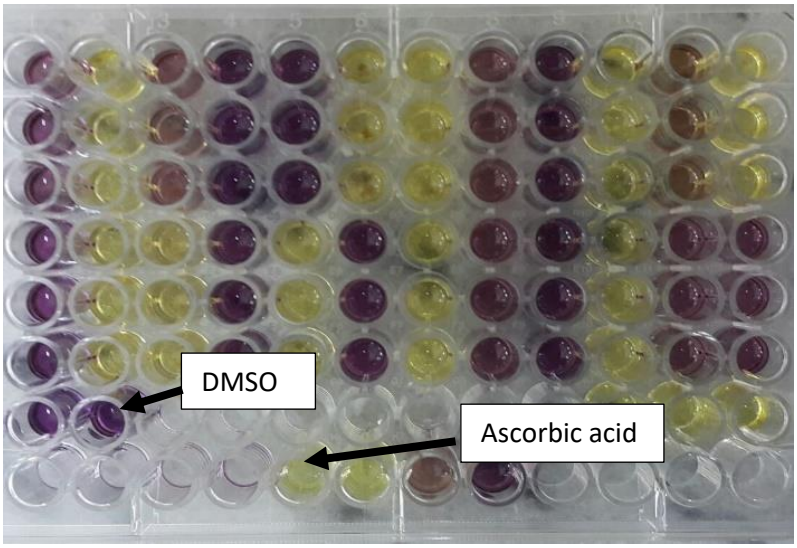

Figure 2. DPPH free radical scavenging activity of *F. benghalensis* extracts.

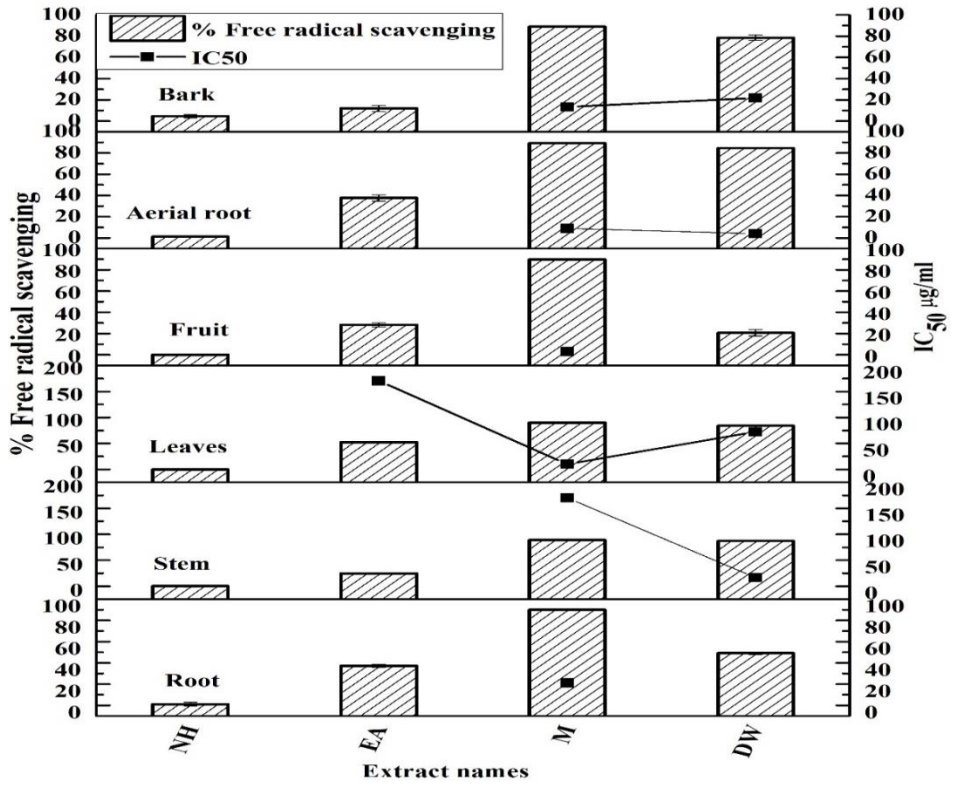

Figure 3. DPPH free radical scavenging activity and IC50 of F. benghalensis extracts. Values shown are mean of triplicate ± standard deviation.

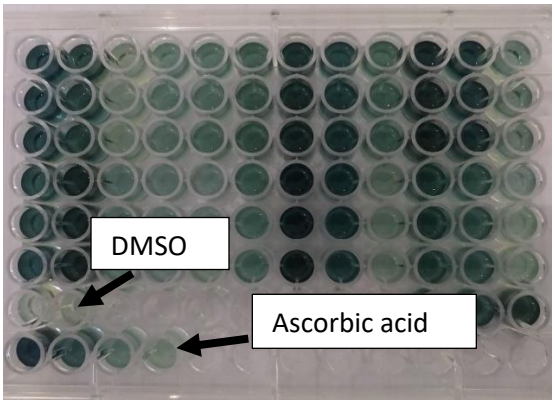

Figure 4. Total antioxidant capacity of *F. benghalensis* extracts.

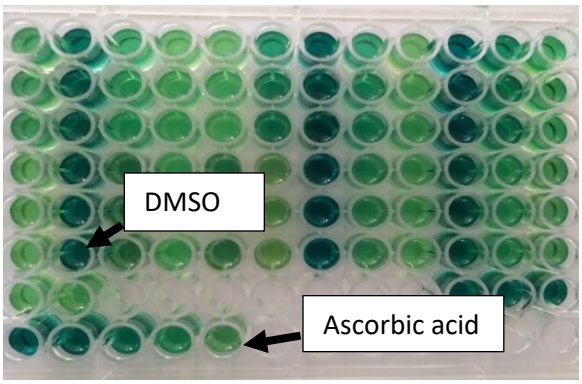

Figure 5. Total reducing power estimation of *F. benghalensis* extracts.

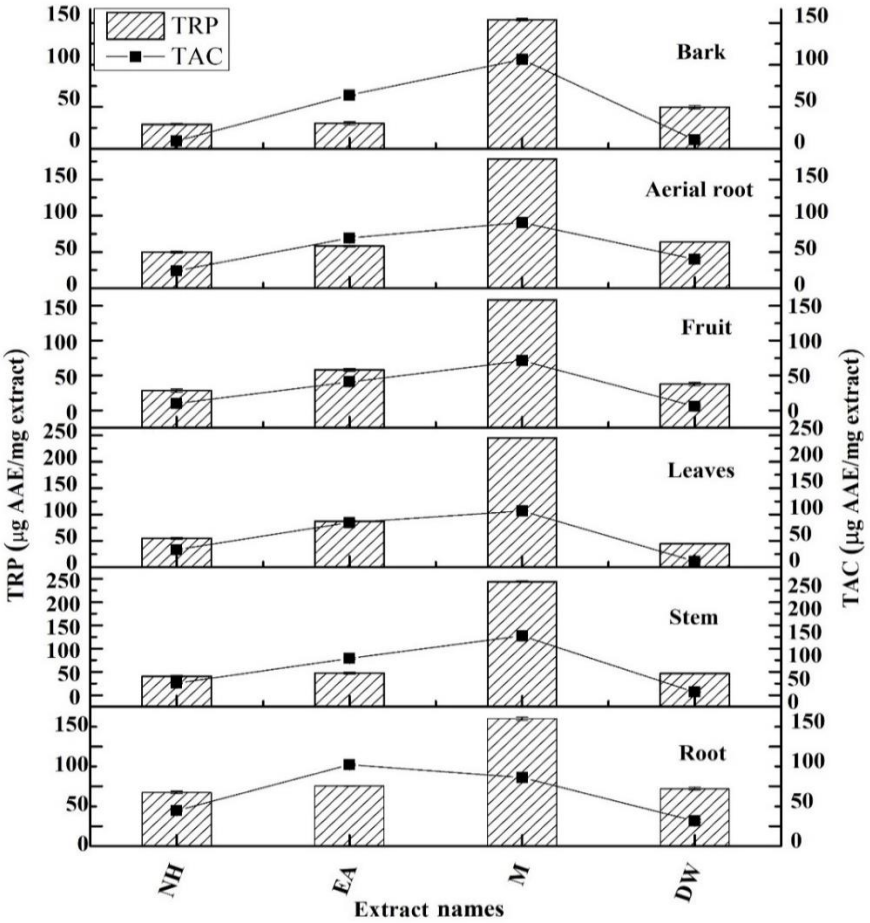

Figure 6. TAC and TRP of *F. benghalensis* extracts. Values shown are a mean of triplicate ± standard deviation.

The phytochemical analysis of different extracts of *F. benghalensis* was conducted by colorimetric assays for the assessment of flavonoids and phenolics. Phenolics are present abundantly in plants having aromatic ring with one or more hydroxyl groups. Maximum gallic acid equivalent phenols in *F. benghalensis* were shown by M extract of stem. It was observed that polar solvents were more efficient in extraction of polyphenols and current results strengthen previous studies (Manian *et al.*, 2008). Solubility of phenolics mainly depend upon polarity. Maximum level of polyphenols have also been reported from other species of *Ficus* such as *F. religinosa* L. and *F. carica* L. (Uma *et al.*, 2009). The phenolics in medicinal plants possess significant pharmacological activities such as antibacterial, antiviral, antitumor, anthelmintic and antioxidant. Antioxidant potential is due to presence of various functional groups such as hydroxyl, ketonic, methoxy and double bond conjugation (Yadav and Agarwala, 2011). Detection of significant phenolic contents in *F. benghalensis* propose it as an endless source of natural antioxidant.

Second most abundant secondary metabolite in plants are hydroxylated phenolic substances called flavonoids. They are responsible for antioxidant properties due to presence of hydroxyl group. Antioxidant properties play important role in reduction of oxidative stress, cytotoxicity and strengthen oxidative defense by scavenging free radicals (Chang *et al.*, 2002). Maximum TFC was shown by EA extract of leaves. The possible reason is the solubility of flavonoids in medium polarity solvents. Other species of *Ficus* such as *F. religiosa* also show maximum flavonoids in EA extract (Sultana *et al.*, 2009).

Antioxidant assays

Oxidation in biological systems is a natural phenomenon which results in formation of highly reactive peroxyl and hydroxyl radicals. These radicals ultimately cause damage to DNA, protein and polyunsaturated fatty acid residues of cell membrane and lead to pathological effects such as cancer and vascular diseases. Immune system inactivates the reactive species but the overburden of radicals needs exogenous supply of antioxidants. Antioxidants are integral part of plants as secondary metabolites which play important role as free radical scavengers and by converting highly reactive free radicals into less reactive species (Moon and Shibamoto, 2009). Different studies verify the significant role of antioxidants in reduction of oxidative stress, which provoke us to determine the antioxidant potential of various extract of *F. benghalensis*. The antioxidant capability of samples cannot be assessed by a single assay. Therefore, DPPH free radical scavenging, total reduction power and TAC assays were performed to verify the antioxidant potential.

DPPH is the stable free radical and antioxidant potential of crude extract was determined on the basis of scavenging of free radical i.e. DPPH. The principle of the assay is based on the conversion of purple color of the free radical to the yellow color molecule by accepting a hydrogen electron from donor antioxidants present in samples (Floegel *et al.*, 2011). In the current study, high percentage free radical scavenging activity was shown by M extract of fruit. Various studies expressed linear relationship between TPC and reducing activity (Roy *et al.*, 2010). Significant TPC was also detected in M extracts, which might be

responsible for free radical scavenging activity.

TAC of various samples of *F. benghalensis* was determined by phosphomolybdenum based calorimetric assay. The principle of the assay is based on reduction of Mo (VI) to Mo (V) by natural antioxidant and formation of green color complex which show maximum absorption at 695 nm (Pellegrini *et al.,* 2003). The highest TAC was expressed by M extract of stem. Previous studies also indicated that M extract showed maximum antioxidant activity due to presence of polyphenols in polar solvents. Studies indicate positive correlation between polyphenols and TAC (Kumaran and Karunakaran, 2007).

Total reducing power is based on the presence of reductones which are associated with antioxidant activity by breaking the radical chain by donating a hydrogen atom (Wong *et al.,* 2006). Maximum reducing power was shown by the M extract of stem. Studies performed on certain plant extracts proved that polyphenols and reducing power has direct correlation so results were in agreement with the previous findings (Ou *et al.*, 2002).

CONCLUSION

The phytochemical analysis exhibited highest TPC in M extract of stem and TFC in EA extract of leaves i.e. 61.2±1.3 µg GAE/mg extract and 25.1±0.9 µg QE/mg extract respectively.

Total reduction power and TAC were maximum in the M extract of stem i.e. 243.89±1.6 µg AAE/mg extract and 127.08±2.7 µg AAE/mg extract respectively.

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