Antioxidant activity of *Artocarpus altilis* (Parkinson) Fosberg leaves

Mukesh S. Sikarwar*, Boey Jia Hui, Kumutha Subramaniam, Bavani Devi Valeisamy, Ling Kar Yean, Kaveti Balaji

Unit of Pharmaceutical Chemistry, Faculty of Pharmacy, AIMST University, Semeling, Kedah Darul Aman, Malaysia

ABSTRACT

Objective: This research was aimed to investigate the antioxidant activity of *Artocarpus altilis* (Parkinson) Fosberg leaves. **Materials and Methods:** 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and thiobarbituric acid (TBA) methods were used to determine the antioxidant activity with ascorbic acid as a reference. **Results:** Total phenolic content was found to be 26.22 mg gallic acid equivalents/g. In DPPH assay, the maximum free radical scavenging activity of the extract was found to be 70.59%. The TBA method also supported the antioxidant potential of the plant extract. **Conclusion:** From this detailed study, it can be concluded that the ethanolic extracts of *A. altilis* leaves possess moderate antioxidant property. It may be due to the presence of a modest amount of phenolic compounds in the leaves of this plant. Hence, the ethanolic extract of this plant may not act as first-line antioxidant defense but may be used as supportive antioxidant agent.

Key words: Antioxidant activity, *Artocarpus altilis*, 2,2 diphenyl-1-picrylhydrazyl

INTRODUCTION

Herbal medicine

Herbal medicine in the simplest form is the medicines or drugs made up from herbs or plants, and can be said to process several synonyms all of that refers to plants as the raw materials for medicine namely, phytomedicines, plant medicines, green medicines, traditional medicines, traditional remedies plant drugs, and forest health products among others.

In this modern setting, ingredients are sometimes marketed for uses that were never considered in the traditional healing systems from, which they emerged. Use of ephedra for weight loss or athletic performance enhancement is one of the examples. Meanwhile in some countries, herbal medicines are subjected to rigorous manufacturing standards, which is not seen everywhere. In Germany, herbal products are sold as "phytomedicines," in which they are subjected to the same criteria for efficacy, safety and quality as are other drug products. In USA, most herbal products are sold as dietary supplements that do not require any pre-approval on these criteria basis.[1-4]

***Corresponding address:**
Dr. Mukesh S. Sikarwar, Faculty of Pharmacy,
AIMST University, Semeling, 08100 Bedong,
Kedah Darul Aman, Malaysia.
E-mail: mukeshsikarwar@gmail.com

DOI: 10.5530/fra.2014.2.7

Artocarpus altilis

A. altilis (Moraceae) is a tropical fruit, and the tree produces fruit twice in a year with some fruiting throughout the year.[5] Breadfruit is also known for a traditional starch rich crop. The genus *Artocarpus* (Moraceae) contains nearly 50 species, and is commonly distributed in tropical and subtropical regions.[6] The generic name of the species comes from the Greek words "artos" (bread) and "karpos" (fruit) and the fruits eatable are commonly called as "breadfruit".[7] Synonyms of *A. altilis* are *Artocarpus communis* and *Artocarpus incisus*.[8] Leaves are leathery with a dark-green color on the dorsal side, which often appears to be glossy. The underside is dull with an elevated midrib and main veins. Leaves are alternate and distichous, entire to pinnate, coriaceous, glabrous to pubescent. At the end of the branches, the leaves are seen as clusters. The crown is conical in shape, when the trees are younger or grown

under shaded condition, and they become rounded and irregular, when it turns older. Basically, *Artocarpus* species consists of phenolic compounds which include flavonoids, stilbenoids, arylbenzofurons and Jacalin, a lectin. Over 130 compounds are identified in various organs of the tree of *A. altilis*, more than 70 of which derived from the phenylpropanoid pathway.[9]

Leaves of *A. altilis* contain tannins, phenolics, glycosides, saponins, steroids, terpenoids and anthraquinones. Flavonoids are present in the petroleum ether and ethyl acetate leaf extracts, whereas tannins are detected only in the methanol leaf extract. It also contains phenolic compounds such as stilbenes, chalcones and flavones.[10] The leaves of selected plant are illustrated in Figure 1.

The extracts and metabolites of *Artocarpus* are used in the various biological activities including antiviral, antifungal, antiplatelet, anti-arthritic, antibacterial, anti-tubercular, tyrosinase inhibitory and cytotoxicity.[11] The fruits are a great source of carbohydrate, and it has low fat. Since, the fruit can be steamed, fried, baked, roasted and fried, they can be eaten at all stages of growth. Although breadfruits are rich in carbohydrates, however they are substituted partially by wheat flour in many products such as snacks, pastries, and bread.

Taxonomical classification[12]

Kingdom:	Plantae
Subkingdom:	Mracheobionata
Division:	Magnoliophyta
Class:	Magnoliopsida
Subclass:	Hamamelididae
Order:	Rosales
Family:	Moraceae
Genus:	*Artocarpus*
Species:	Altilis

Uses

Pharmacological uses[13]

Many on-going researches are testing the pharmacological activities of *A. altilis*. Some of the researches that are being carried out based on these plants include anti-inflammatory, antioxidant, antifungal, immunomodulatory effect, antidiabetic effect, antibacterial effect, anti-cholinergic, nutritional assessment, cosmetic agent, ace inhibitors, anthelmintic effect, protease inhibitors, regulation of estrogens, and inhibition of melanin biosynthesis.

Traditional uses[14]

The senescence leaves are used in the treatment of hypertension and diabetes in Caribbean. In Taiwan, leaves are used in the treatment of liver disease and fever. Roasted leaf powder is used as a remedy for enlarged spleen.

Interactions[15]

5-Alpha reductase inhibitors

Based on laboratory tests, heartwood extract of *A. altilis* may produce a potent 5-alpha reductase inhibitory activity; and care must be taken when using 5-alpha reductase inhibiting herbs and supplements, as the additive effects may cause some adverse reactions.

Anticoagulants and anti-platelets

Based on an *in vitro* study, root extracted from *A. altilis* can inhibit the formation of thromboxane (an inducer of platelet aggregation). According to the *in vitro* studies conducted on animals and humans, frutackin shows hemagglutination activity against erythrocytes and therefore, they may have additive effects with antihemagglutinin in herbs or supplements.

MATERIALS AND METHODS

Collection

Fresh leaves of *A. altilis* were collected in the month of March 2014 in and around campus of AIMST University, Kedah, Malaysia. The leaves were separated, washed thoroughly, shade dried for 7 days, and also dried using hot air oven to quicken the drying process. Then, the leaves were homogenized to a fine powder using electronic blender and stored in air tight container.

Standardization of plant material

The plant was macroscopically examined for shape, size, surface, characteristic, texture, color, odor, and taste. Powder microscopy was carried out to check authenticity of the plant material.

Drug was subjected to physical and chemical evaluation for different parameters e.g. loss on drying, ash values, thin layer chromatography (TLC), and fluorescence analysis. Determination of alcohol-soluble, water-soluble, chloroform-soluble and petroleum ether-soluble extractive were carried out as per the methods described in official literature.[16-22]

Extraction[23,24]

Soxhlet apparatus was used for extraction. The extract was concentrated using a rotary evaporator and stored in the refrigerator for further use.

Phytochemical screening[25]

Phytochemical screening was carried out using standard methods as described in reference literature and reference text.

Total phenolic content[26]

Gallic acid stock solution (1000 mg/ml) was prepared by dissolving 100 mg gallic acid in 100 ml ethanol. Many dilutions of standard gallic acid were arranged from this stock solution. Folin–Ciocalteau reagent was prepared by mixing Folin's reagent with phenol reagent (1:1) and diluted 1:1 in distilled water, before use. Calibration curve was plotted by mixing 1 ml aliquots of 1, 2, 3, 4, 5 and 6 mg/ml of gallic acid solutions with 5 ml of Folin–Ciocalteau reagent and 4 ml of sodium carbonate solution. The absorbance was measured after 30 m at 765 nm. Stock solution of the extract was prepared by dissolving 10 mg of extract in 100 ml of 95% ethanol. The concentration of the ethanol extract stock solution is 100 μg/ml. The stock solution of ethanol extract was diluted to 50 μg/ml. 1 ml of ethanol extract (50 μg/ml) was mixed separately, with the same reagent as what was done in construction of calibration curve, and after 1 h, the absorbance was measured for determination of total phenolic compound in both the extract separately using the formula:

Total phenolic content, C = A/B

C - Expressed as mg gallic acid equivalents (GAE)/g dry weight of extract.

A - Equivalent concentration of gallic acid established from the calibration curve (mg).

B - Dry weight of extract.

Antioxidant studies[27-29]

2,2 diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay

DPPH solution is also prepared by dissolving 19.7 mg of DPPH in 500 ml of 95% of ethanol. The concentration of DPPH solution is 0.1 mM. Stock solution of the extract was prepared by dissolving 10 mg of the extract in 100 ml of 95% ethanol. The concentration of the stock solution of the extract was 100 μg/ml. Different dilutions of extract (40, 60, 80, 100 μg/ml) were prepared. Another stock solution of the extract was prepared by dissolving 10 mg of the extract in 10 ml of 95% ethanol. The concentration of this stock solution is 1000 μg/ml. From this 1000 μg/ml of stock solution, different dilutions (150 and 200 μg/ml) were prepared. Then 2 ml of the extract from each dilution (40, 60, 80, 100, 150, 200 μg/ml) was added into the test tube containing 2 ml of DPPH solution. The mixture was shaken strongly and kept at room temperature for 30 m in the dark. Then, the absorbance was measured at 515 nm using ultraviolet-visible spectrophotometer. Lower absorbance of the mixture indicates higher free radical scavenging activity. Positive control or standard was prepared. Ascorbic acid was used as a control or a standard. Different dilutions of ascorbic acid solutions (2, 4, 6, 8, 10, 12 μg/ml) were prepared. Blank solution was prepared. The blank solution contained 2 ml of 95% ethanol and 2 ml of DPPH solution without any extract or ascorbic acid solution.

% Scavenging activity = ([Ac-As]/Ac) × 100%
Ac - Absorbance of the control reaction
As - Absorbance of the sample of the extracts

The antioxidant activity of the extract was expressed as inhibitory concentration (IC_{50}). The IC_{50} value is defined as the concentration of the extract that inhibits the formation of DPPH radicals by 50%. The IC_{50} values were calculated from the graph by linear regression analysis.

Thiobarbituric acid (TBA) assay

TBA method, described by Ottolenghi was followed: The final *A. altilis* ethanolic extract at concentration of 50 μg/ml and 100 μg/ml were used in this method. A mixture of 4 mg of sample (50 μg/ml) in 4 ml ethanol, 4.1 ml of 2.51% linoleic acid in ethanol, 8.0 ml 0.02 M phosphate buffer (pH 7.0) and 3.9 ml of distilled water contained in screw cap vial in an oven at 40°C in the dark. 2 ml of 20% trichloroacetic acid and 2 ml of 0.67% of TBA were added to 1 ml of sample solution. The mixture was positioned in a boiling water bath for 10 min and then centrifuged after cooling at 3000 rpm for 20 min. The absorbance activity of the supernatant was measured at 552 nm and recorded after it has reached its maximum. The steps were repeated using 100 μg/ml of the *A. altilis* ethanolic extract. Ascorbic acid was used as a control or standard. Same steps were repeated by replacing the extract with ascorbic acid.

RESULTS

Standardization of plant material

The leaves are alternate or irregular, ovate and finger-like shaped with an average of 30.21 cm in length (20-40 cm) and 24.25 cm in width (17-36 cm). The apexes of the leaves are acute, conical shaped crown with thick petioles having length of 4.2-4.8 cm. The leaves have a bitter

taste with slight odor, and they are dark green in color on the dorsal side and yellowish green on the ventral side. Microscopic features of powder, fluorescence analysis and physicochemical parameters were found to be in agreement with reported and official literature.

TLC showed four different spots at R_f value 0.9, 0.66, 0.48 and 0.34. One of the result of TLC was similar to gallic acid R_f value.The standard R_f value of gallic acid as per British Pharmacopeia is 0.35 ± 0.01. Phytochemical analysis also confirmed the presence of tannin and phenolic compound, anthraquinone glycoside and flavonoids.

Total phenolic content

Total phenolic content was found to be 26.2 GAE/g. The graph of total phenolic content is illustrated in Figure 2.

Figure 1: *Artocarpus altilis* (Parkinson) Fosberg leaves

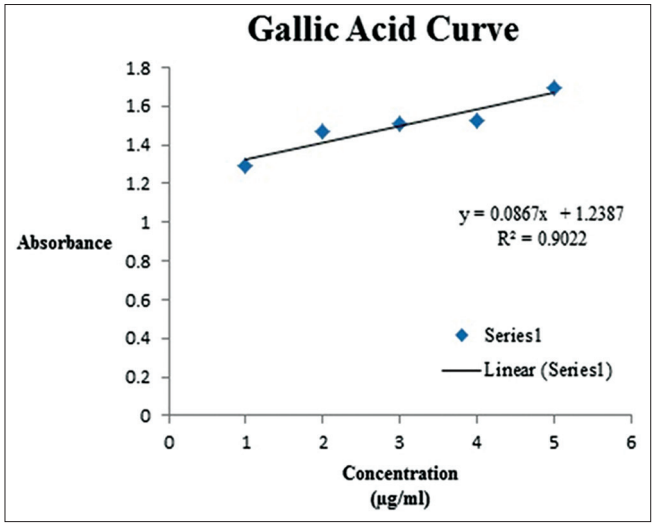


Figure 2: Total phenolic content of *Artocarpus altilis* (Parkinson) Fosberg leaves

Antioxidant study

DPPH scavenging activity

IC_{50} of the ascorbic acid and ethanolic extract of *A. altilis* was found to be 7.532 and 140.54 µg/ml respectively. The graph of DPPH scavenging activity ascorbic acid and ethanolic extract of *A. altilis*is illustrated in Figures 3 and 4.

TBA method

Ethanolic extract of *A. altilis* leaf has lesser absorbance as compared to the absorbance of ascorbic acid, proving that the leaf extract possesses lesser antioxidant activity than the ascorbic acid. The graph of TBA method of ascorbic acid and ethanolic extract of *A. altilisis* illustrated in Figure 5.

DISCUSSION

The estimation of total phenolic content was based on the absorbance of sample and Folin-Ciocalteu reagent mixture

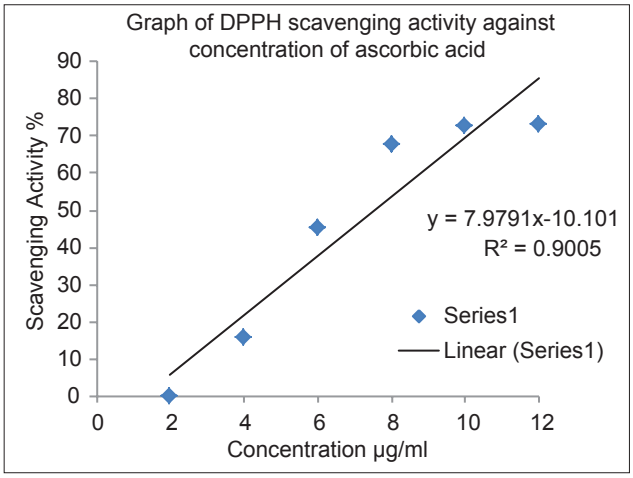


Figure 3: 2,2 diphenyl-1-picrylhydrazyl scavenging activity of ascorbic acid

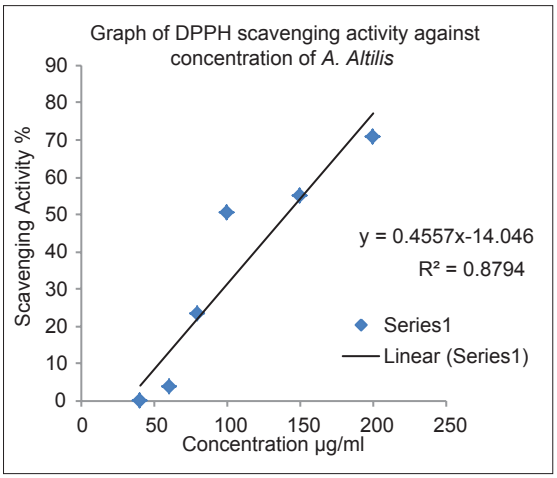


Figure 4: 2,2 diphenyl-1-picrylhydrazyl scavenging activity of ethanolic extract of *Artocarpus altilis* leaves

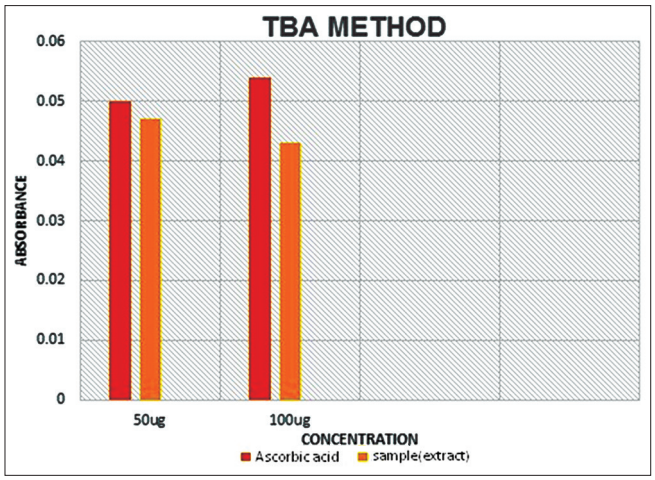


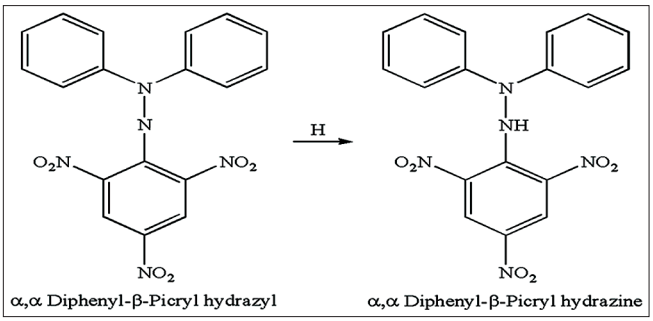
Figure 5: Antioxidant activity of ascorbic acid and ethanolic extract of *Artocarpus altilis* leaves in thiobarbituric acid method

at 765 nm. Gallic acid was used as a standard compound and the total phenols were expressed as μg/ml gallic acid equivalent using the standard curve equation: $y = 0.0079x + 1.476$, $R^2 = 0.9949$, where y is the absorbance at 765 nm and x is the total phenolic content expressed in μg/g. The phenolic content in the ethanolic extract was found to be 26.22 GAE/g. Antioxidant activity of plant extract was mainly due to presence of phenolic compound that may exert an antioxidant effect as free radical scavengers. *A. altilis* showed moderate free radical scavenging activity which might be due to their low phenolic constituents.

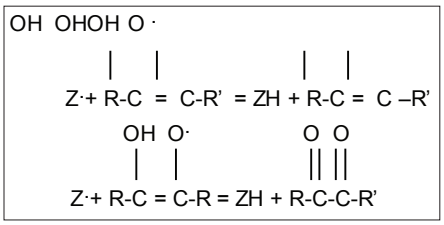
In DPPH scavenging activity method, DPPH is used as a stable free radical. It can cause delocalization of the unused electron over the molecule as a whole so the molecules do not dimerise. The delocalization also gives rise to the deep violet color. Absorption band in ethanol solution was measured at 515 nm. When a solution is mixed with a substance that have antioxidant properties, the substances will donate a hydrogen atom then this give rise to the reduced form with the loss of violet color; and the color will turn to pale yellow. The color is turned to pale yellow, due to the presence of the picryl group. The reaction can be represented as follow:

$X^{\cdot} + ZH = XH + Z^{\cdot}$

$X^{\cdot}$ is the DPPH radicals, and ZH is the substances that have antioxidant properties and can reduce DPPH radicals. XH is the reduced form, and $Z^{\cdot}$ is the free radicals. These radicals will further undergo reaction that controls the overall stoichiometry that is the number of molecules of DPPH reduced by one molecule of reductant. Therefore, the activity of DPPH radicals can be suppressed by the substances ZH.



In this study, ascorbic acid was used as a standard drug or reference, which was compared with the ethanol extract of selected plant. Ascorbic acid has two adjacent sites for hydrogen abstraction. Therefore, there will be further abstraction reaction after the first one. This leads to 2:1 stoichiometry that is two molecules of DPPH reduced by one molecule of ascorbic acid. Therefore, ascorbic acid has a strong antioxidant activity. The reaction is as below:



IC_{50} or efficient concentration is defined as the concentration of the substrate that causes 50% loss of the DPPH activity. The higher the antioxidant activity, the lower is the value of IC_{50}.

Our detailed study results showed that when we increased the concentration of the extract which is from 40 μg/ml to 200 μg/ml, the absorbance value was decreased so far. As a rule, when the absorbance value is decreased, the antioxidant activity of the extract is increased. The purple color solution of DPPH turns yellow after getting reduced by the extract and ascorbic acid. This gives us evidence that our ethanolic extract possesses antioxidant properties. We assume that when the concentration of the extract increased from 400 to 800 μg/ml, the absorbance value was increased and thus the antioxidant activity of the extract was reduced. When concentration of the extract increased, more complex nature of the extract hinders the antioxidant activity. Maximum scavenging activity of the ascorbic acid at the concentration of 12 μg/ml was 73.11%. While the maximum scavenging activity of the extract at the concentration of 200 μg/ml was 70.59%. By comparing the scavenging activity of ascorbic acid and extract, we can assume that ascorbic acid has more antioxidant activity than the extract because the ascorbic acid has more free radical scavenging potential at low concentration as compared to

the extract. However, the extract has moderate antioxidant activity at high concentration. IC_{50} of the ascorbic acid was 7.532 μg/ml while the IC_{50} of the extract was 140.54 μg/ml. The lower the value of the IC_{50} is, the higher the antioxidant activity. The ascorbic acid has higher antioxidant activity compared to the ethanol extract. From this study, we can conclude that *A. altilis* leaves extract has moderate antioxidant activity.

In TBA method, ethanol extract of *A. altilis* leaf were examined for their ability to act as radical scavenging agents in comparison to ascorbic acid through the TBA reactive substance TBA method. The TBA assay procedure measures the extent of lipid degradation by reacting TBA with malondialdehyde (MDA) and other secondary lipid peroxidation product which then forms a pink pigment TBA when measured spectrophotometrically at the absorbance of 552 nm. It is assumed that the formation of MDA from fatty acids below three double bonds occurs via the secondary oxidation of primary carbonyl compounds. In this study, it was found that the ethanolic extract of *A. altilis* leaf have lesser absorbance as compared to the absorbance of ascorbic acid, suggesting that the leaf extract possesses much less antioxidant activity than the ascorbic acid.

CONCLUSION

A. altilis plant leaves were selected for this study. The method used for extraction of *A. altilis* was soxhlet extraction method. All the standardization parameters for the leaves of selected plant were done according to the pharmacopoeia standards, and results of these parameters were found within the limits. Based on the studies conducted, it was confirmed that the plant contains flavonoid, and glycosides. Phenolic compound was also present in the alcoholic extract. The antioxidant activity was done using DPPH and TBA methods. *A. altilis* leaves ethanolic extract showed moderate antioxidant activity as compared to ascorbic acid. From this paramount study, it can be concluded that the *A. altilis* leaves extracts contain moderate antioxidant property as compared to ascorbic acid. It may be due to the presence of a modest amount of phenolic compounds in the leaves of the plant. Hence, the ethanolic extract of this plant may not act as first-line antioxidant defense but may be used as a supportive agent.

In future, further investigations are required to isolate the chemical constituent responsible for its antioxidant activity. This plant is proven to have antioxidant potential, hence further investigation on various pharmacological activities supported by antioxidant potential is recommended.

REFERENCES

1. Osmene KP, Elujoba AA, Ilori MO. An overview of herbal medicine research and development in Nigeria. Res J Med Sci 2011;5:228-32.
2. An introduction to herbalism. Healthy Life J. Available from: http://www.healthylifejournal.org/articles/an-introduction-to-herbalism/. [Last accessed on 2014 Apr 22].
3. History of Use of Traditional Herbal Medicines. IARC Monographs. Available from: http://www.monographs.iarc.fr/ENG/Monographs/vol82/mono82-6A.pdf. [Last accessed on 2014 Apr 22].
4. Challenges and Future Directions of Herbal Medicine Research. Explore IM Integrative Medicine. Available from: http://www.exploreim.ucla.edu/research/challenges-and-future-directions-of-herbal-medicine-research/. [Last accessed 2014 Apr 22].
5. Akanbi TO, Nazamid S, Adebowale AA. Functional and pasting properties of a tropical breadfruit (*Artocarpus altilis*) starch from Ile-Ife, Osun State, Nigeria. Int Food Res J 2009;1:151.
6. Motley TJ. Breadfruit origins, diversity and human facilitated distribution. Available from: http://www.herbarium.millersville.edu/325/Zerega-2005.pdf. [Last accessed on 2014 Jan 21].
7. Jones AM, Ragone D, Tavana NG, Bernotas DW, Murch SJ. Beyond the bounty: Breadfruit (*Artocarpus altilis*) for food security and novel foods in the 21st century. Ethnobotany J 2011;9:131-2.
8. Orwa C, Mutua A, Kindt R, Jamnadass R, Simons A, 2009. *Artocarpus altilis*. Agroforestree Database: A tree reference and selection guide version 4. Available from: http://www.worldagroforestry.org/af/treedb/ [Last accessed on 2014 Jan 21].
9. Handa SS, Khanuja SP, Longo G, *et al*. Extraction technologies for medicinal and aromatic plants. International Centre for Science and High Technology; 2008. Available from: http://www.agritech.tnau.ac.in/horticulture/extraction_techniques%20_medicinal_plants. Pdf [Last accessed on 2014 Feb 03].
10. Amarasinghe NR, Jayasinghe L, Hara N, Fujimoto Y. Chemical constituents of the fruits of *Artocarpus altilis*. Biochem Syst Ecol 2008;36:323-5. Available from: http://www.sciencedirect.com/science/article/pii/S0305197807002013. [Last accessed on 2014 Feb 01].
11. Jagtap UB, Bapat VA. Artocarpus: A review of its traditional uses, phyto chemistry and pharmacology. J Ethnopharmacol 2010;12:143-4. Available from: http://www.elsevier.com/locate/jethpharm. [Last accessed on 2014 Jan 21].
12. Sushmita, Nayeem N. *Artocarpus altilis*: Over view of a plant which is referred to as bread fruit. Int J Pharm Sci Lett 2013;3:273-6.
13. Somashekhar M, Nayeem N, Sonnad B. A review on family *Moraceae* (Mulberry) with a focus on *Artocarpus* species. World J Pharm Pharm Sci 2013;2:2614-21.
14. Morton JF. Fruits of Warm Climates. United State: Julia F. Morton Publishing; 1987.
15. Lassens Natural Foods and Vitamins. Breadfruit (*Artocarpus altilis*). Available from: http://www.naturalstandard.com. [Last accessed on 2014 Jan 21].
16. Arya V, Thakur R. Organoleptic and microscopic analysis of *Gentinaregeliana*. J Pharmacogn Phytochem 2012;1:32-5.
17. Fuloria SS, Pahwa S, Kumari S, Gupta SK. Studies on morpho-microanatomical evaluation of the leaves of *Tridax procumbens* linn (Astteraceae). J Sci Res 2010;2:613-9.
18. Bharat J. Determination of quality standards for herbal formulation: Chaturjat Churna. J Pharmacogn Phytochem 2013;2:103-4.
19. Tambe SS. Determination of ash values of some medicinal plant of Marathwada region in Maharashtra. Int J Pharm Res Biosci 2012;1:337-46.
20. Thirumalai D. Evaluation of physiochemical, pharmacognostical and phytochemical parameters of *Premna herbacea*. Asian J Pharm Clin Res 2013;6:173-81.
21. Pradhan C. Assessment of the antibacterial potential of breadfruit leaf extracts against pathogenic bacteria. Int J Pharm 2013;3:374-9.
22. Thin Layer Chromatography Procedure. Available from: http://www.amrita.vlab.co.in/?sub=3&brch=63&sim=154&cnt=2. [Last accessed on 2014 Apr 21].
23. Beverly Swaile. Soxhlet extraction of fat from French fries. Available from:

http://www.slideshare.net/princeelackad/Soxhlet-extraction. [Last accessed on 2014 Apr 21].

24. Hot Maceration Extraction Procedure. Available from: https://www.workspace.imperial.ac.uk/library/Public/Vancouver_referencing.pdf. [Last accessed on 2014 Apr 21].
25. Khandelwal KR. Preliminary phytochemical screening. Practical Pharmacognosy; 2005. p. 146-57. Available from: http://www.allbookez.com/kokate-practical-pharmacognosy/[Last accessed on 2014 Jan 21].
26. Sharma GN. Phytochemical screening and estimation of total phenolic content in *Aeglemarmelos* seeds. Int J Pharm Clin Res 2011;3:27-9.
27. Ottolenghi A. Interaction of ascorbic acid and mitochondria lipids. Arch Biochem Biophys 1959;79:355-63.
28. Molyneux P. The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol 2003;26:211-9.
29. Bondet V, William B, Berset C. Kinetics and mechanism of antioxidant activity using the DPPH free radical method. Lebensm Wiss Technol/Food Sci Technol 1997;2:601-9.