Journal of Advances in Biology & Biotechnology

Volume 27, Issue 8, Page 661-667, 2024; Article no.JABB.120130
ISSN: 2394-1081

Phytochemical Analysis of *Agathis robusta* Using Ethyl Acetate

Tanu Tyagi [a*], Y. Vimala [a++], Ishwar Singh [a#], Anamish Tyagi [b†] and Archasvi Tyagi [c†]

[a] Department of Botany, CCS University, Meerut Campus, Uttar Pradesh, India.
[b] Department of Genetics and Plant Breeding, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh, India.
[c] C.C.S University, Meerut Campus, Uttar Pradesh, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jabb/2024/v27i81181

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/120130

Original Research Article

Received: 20/05/2024
Accepted: 22/07/2024
Published: 30/07/2024

ABSTRACT

It is often recognized that India is home to a diverse range of biological species. For this study, *Agathis robusta* were employed. The present investigation aimed to determine the total phenolic and flavonoid contents of the selected plants and investigate the presence of phytochemicals. stems and leaves of A. robusta were collected, dried, and ground into a fine powder. The powdered leaves were extracted using a Soxhlet method and ethyl acetate. The finished extract was concentrated while the pressure was decreased to produce a crude extract. To determine the amount of sugar, carotenoid, and total protein in the crude extract, a phytochemical screening was conducted using standard qualitative procedures. A. robusta leaf ethyl acetate extract showed the presence of several phytochemicals. Significant amounts of terpenoids, flavonoids, alkaloids, and tannins were found in the extract. These substances' existence suggests that they may have

[++]Senior Professor;
[#]Assistant Professor;
[†]Ph.D. Scholar;
**Corresponding author: E-mail: tanutyagi055@gmail.com;*

Cite as: *Tyagi, Tanu, Y. Vimala, Ishwar Singh, Anamish Tyagi, and Archasvi Tyagi. 2024. "Phytochemical Analysis of Agathis robusta Using Ethyl Acetate". Journal of Advances in Biology & Biotechnology 27 (8):661-67. https://doi.org/10.9734/jabb/2024/v27i81181.*

pharmacological effects, such as antibacterial, anti-inflammatory, and antioxidant capabilities. The ethyl acetate extract of A. robusta leaves revealed the presence of several phytochemicals. The extract included notable concentrations of tannins, alkaloids, flavonoids, and terpenoids. The presence of these compounds raises the possibility that they have pharmacological properties including antioxidant, anti-inflammatory, and antibacterial properties.

Keywords: Phytochemical analysis; Araucariaceae; biological species.

1. INTRODUCTION

One of the oldest coniferous trees in the Araucariaceae family, *Agathis robusta* (C. Moore ex F. Muell) Bailey [1], is endemic to the Bismarck Archipelago, New Guinea, and Queensland. It is sometimes referred to as Kauri pine, Queensland Kauri, or smooth-barked Kauri [2]. Primarily referred to by its popular names, Kauri pine or Queensland kauri, it is among the oldest living things on Earth. Still, just a few countries in the Southern Hemisphere still harbor it, including Australia, where it originated, India, Malaysia, and Papua New Guinea [2,3].

This monoecious evergreen plant has smooth bark and grows up to 50 meters tall, straight branches, and other distinguishing features from a botanical perspective. With no midrib, the leaves are elongated and wide, arranged in opposing pairs above the stem. It yields two distinct cones: the enormous, globose seed-producing female cones fracture to release the seeds when they reach maturity. Conversely, the pollen is made up of male cones that are quite tall and thick, with a cylindrical form [4].

A. robusta has been the subject of several literary works. Their primary areas of interest ranged from botany [4] [5] to the content of diterpenoids (Carman & Dennis 1964; Carman et al. 1973) and the makeup of essential oils [6,3]. Furthermore, some studies on the pharmacological characteristics of this species have reported that the ethanolic extract of the leaves has a good anti-inflammatory activity [7], and that the essential oil has an intriguing antibacterial effect [3]. This is also supported by the essential oil obtained from the leaves of another species in the genus, *A. dammara* (Lamb.) Rich (Zhifen et al. 2015).

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Material

Fresh leaves and stem segments were gathered at the C.C.S. University Meerut campus's botany department. After cleaning the various portions of *A. robusta* to remove dirt and other contaminants, they were dried for 48 hours at 60°C in the oven and ground into a powder using an electronic blender. For the purpose of extracting the solvent from each sample, powder was sieved and then piled into a sealed container.

2.2 Moisture Percentage Analysis

Using Digital Balance (OHAUS Adventure), the fresh weight (Wt.) and dry weight (Wt.) of various A. robusta (kauri pine) pieces were evaluated. Their moisture percent was then determined. First, all of the plant pieces that had been gathered were cleaned with water to get rid of any dust and then dried. determined their fresh weight and then placed them in paper envelopes with clear labels before storing them in the oven. For 48 hours, the material was dried at 60°C. To ensure dry weight stability, the dried plant pieces were once again dried in an oven for a full day before being weighed again. The method used to calculate moisture was (Fresh weight-Dry weight / Fresh weight × 100 [8].

2.3 Biochemical Analysis

Soxhlet extraction: 2 gm of each plant material was extracted with 250 ml of ethyl acetate for 10 hours at a temperature 77.1^0C in a soxhlet apparatus. The extract was concentrated to 55 ml and used for further study.

2.4 Estimation of Total Phenolic Content

Using Folin & Ciocalteu's phenol reagent, the total phenolic content of extracts from various plant sections was ascertained. For the purpose of total phenolic content measurement, 5 ml plant extract samples (8 mg/ml) were utilized. (50.0 mg of dried material from the thimble was homogenized in a mortar and pestle with 5 ml of 80% ethanol for phenolic extraction, following extraction in ethyl acetate). After another centrifugation at 5,000 rpm, the supernatant was collected. For further examination, the residues were repeatedly extracted using 2.5 milliliters of 80% ethanol. All of the obtained supernatant or extract samples were dried out by evaporating

them completely, and any leftover material was then dissolved in 5.0 milliliters of distilled water. Distilled water was used to dilute aliquots in various amounts to prepare the working standard (0.2, 0.4, 0.6, 0.8, and 1.0 ml) up to a maximum of 3 ml for spectral reading. Next, dilute 0.5 ml of Folin & Ciocalteu's phenol reagent with twice as much distilled water. 2 ml of 20% sodium carbonate (Na2CO3) was added after 3 minutes, and for 1 minute, it was completely mixed in a water bath. After cooling the test tubes, the absorbance was calculated at 650 nm. The blank is made up of 2.0 ml of 20% sodium carbonate (Na2CO3), 0.5 ml of Folin & Ciocalteu's phenol reagent, and 3.0 ml of D.W. According to the calibration curve of Gallic acid equivalent at mg/ml, the total phenolic content was reported in mg/g dry weight of tissue [9].

2.5 Determination of Reducing, Non-reducing and Total sugar

A 50 mg sample of plant material was homogenized in 80% ethanol and left on a water bath until the alcohol was no longer odorous. To get clear supernatant, the extract was centrifuged. Drop by drop, 5.0 ml of saturated lead acetate was added to this in order to precipitate proteins, tannins, and other materials that could obstruct the measurement of sugars. Centrifugation was used to extract the precipitate, and 6.0 ml of saturated Na2HPO4 was added to the supernatant to eliminate any remaining lead.3.0 ml of the clean supernatant that was left over after centrifugation was used to measure the amount of reducing sugars. This extract was produced to a volume of 10.0 ml (using DW). After 5.0 ml of extract and 1.0 ml of IN HCI were hydrolyzed and boiled for 20 minutes, the total amount of sugar was calculated. After cooling the hydrolysate and using IN NaOH to raise the pH to 7.0, 10.0 ml of distilled water was added to the volume. A mixture of 1.0 ml hydrolysate and 1.0 mb alkaline copper tartarate (CuSO4-4g Na,CO4-24g, Na-K Tartarate-12g, anhydrous Na-SO-18g, NaHCO3-16g) was combined, and the final volume was adjusted to 1.0 liter using DW. After 20 minutes in the water bath, the tubes were cooled, and 1.0 ml of arsenomolybdate (which was made up of 25g of ammonium molybdate, 3g of sodium arsenate, and 21.0 ml of sulfuric acid) was added. The mixture was then maintained at 48°C for 24 hours, during which time the clear solution was decanted and utilized. The material developed a blue hue, and its absorbance was measured at A660 nm. Nelson (1952) employed a 1.0 mg/ml stock solution of D-glucose (AR-Qualigens) for the standard curve [10].

2.6 Determination of Total Protein

Following homogenization of 100 mg of fresh tissue in 5.0 ml of tris buffer ph 7.0 (containing 3.025 tris, 0.507 g of MgCl2, and 0.273 g of EDTA in 500.0 ml of DW), the mixture was centrifuged at 5000 rpm at 400C. As a protein extract, the supernatant was used. In place of the plant protein extract, 1.0 milliliter of coomassie brilliant blue dye (made by combining 100 milliliters of coomassie brilliant blue R-250, 100 milliliters of 95% ethanol, and 100 milliliters of 80% orthophosphoric acid 595 nm) was added to 1.0 milliliter of the plant protein extract. Using casein (1.0 mg/ml) as the standard protein, a reference curve was created and represented as mg casein/gf.wt [11].

2.7 Estimation of Total Flavonoids

The ammonium chloride colorimetric technique was slightly modified to determine the sample's total flavonoid concentration. In an ultrasonicator set to room temperature, 500 mg of sample were extracted using 10 ml of 80% methanol. Whatman No. 1 filter paper was used to filter the sample extract. Under the same circumstances, the residues were extracted again. On a water bath set at 60°C, the mixed filtrates were evaporated. A mixture of 1.0 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate, and 1.0 ml of distilled water was combined with 1 ml of extract. The combination was left for forty-five minutes to stand at room temperature. Using a spectrophotometer, the mixture's absorbance was calculated at 415 nm.The blank was made using 1 milliliter of 80% methanol, 1 milliliter of methanol, 10% aluminum chloride, 1 milliliter of potassium acetate, and 1 milliliter of distilled water.The total quantity of quercetin was expressed as mg/gm fresh weight based on a calibration curve that was made with µg/ml of quercetin as the standard [12].

2.8 Estimation of Carotenoid content

Jensen, A. [10] Using a pinch of Na2CO3 in the medium, 50 mg of fresh leaf material was homogenized in 5.0 ml of cold 80% acetone (20 ml D.W + 80 ml acetone). The extract was centrifuged for five minutes at 5,000 rpm, and the supernatant was taken out. After 5.0 ml of

final volume was created, absorbance was measured at 480 and 510 nm using 80% acetone as a blank. Following formula was used for calculation:

$$\text{Carotenoid (mg/gmf.wt.)} = 7.6(A_{480})-1.49(A_{510})\times V/1000\times W\times \alpha$$

Where,

V= Final volume of Carotenoid content.
A- Absorbance at specific wavelength.
W-Fresh weight of tissue extracted.

2.9 Estimation of Chlorophyll content

Jensen A. [12] 50 mg fresh leaf material was homogenized in 5.0 ml of chilled 80% acetone (20 ml D.W. +80 ml acetone) using a pinch of Na_2CO_3 in the medium. The extract was centrifuged at 5,000 rpm for 5 min, and supernatant was collected Final volume was made to 5.0 ml and absorbance was read at 645nm and 663 nm against 80 % acetone as blank. Following formula was used for calculation:

$$\text{Chla (mg/gmf.wt.)}=12.7(A_{660})-2.69(A_{645})\times V/1000X\times W$$

$$\text{Chlb (mg/gmf.wt.)}=22.9(A_{645})-4.89(A_{663})\times V/1000\times W$$

$$\text{Total Chl.(mg/gmf.wt.)}=20.2(A645)+8.02(A663)\times V/1000\times W$$

Where

V- Final volume of Chlorophyll extract
A=Absorbance at specific wavelength
W= Fresh weight of tissue extracted

3. RESULTS AND DISCUSSION

The mature leaves of *Agathis robusta* have the highest phenolic content, whereas the mature stem has the lowest. Mature leaves showed the highest level of flavonoids, whereas the mature stem held the lowest amount. Conversely, the mature stem of *Agathis Robusta* had the highest Reducing Sugar Content, while the mature leaf plant portion had the lowest.Finally, total sugar is lower in mature stems and higher in mature leaves. Phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids were found in the plant extracts after analysis.One of the biggest and most common classes of plant metabolites is comprised of phenolic chemicals [13]. They have biological effects that include cardiovascular protection, improvement of endothelial function, antiapoptosis, antiaging, anticarcinogen, anti-inflammation, antiatherosclerosis, and suppression of angiogenesis and cell proliferation [14]. Additionally, they have potent anticancer properties and are powerful antioxidants (Miller et al 1995) [15]. According to Rolland et al. [16], reducing and nonreducing sugars are crucial to the central metabolic pathways and aid in the synthesis of secondary metabolites that improve the therapeutic qualities of plants.

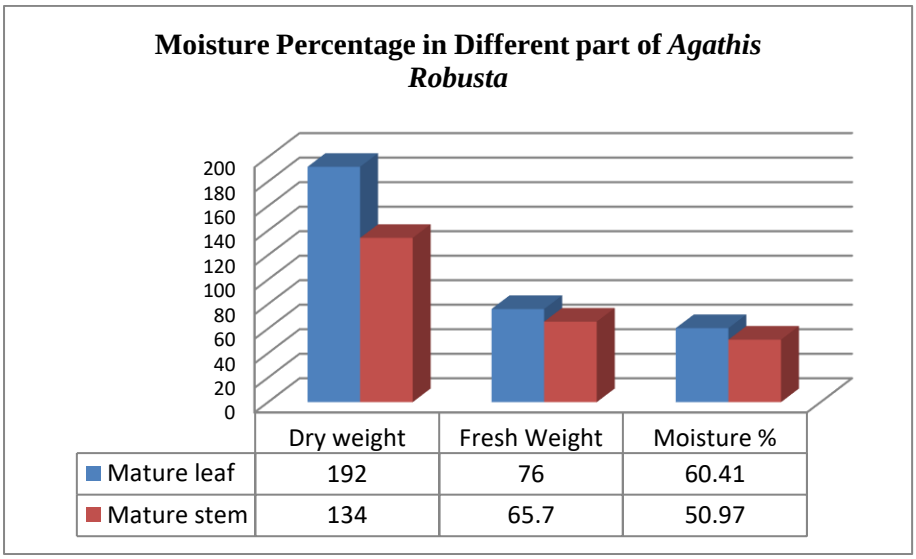

Fig. 1. Maximum moisture percentage was recorded in Mature Leaf and minimum moisture percentage was retained by Mature stem

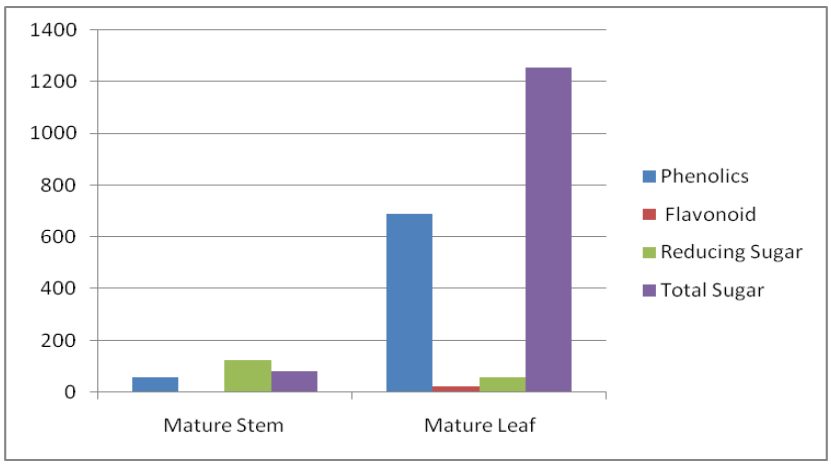

Fig. 2. Phenolic, Flavonoid, Reducing sugar and Total sugar in Different part of *Agathis Robusta*

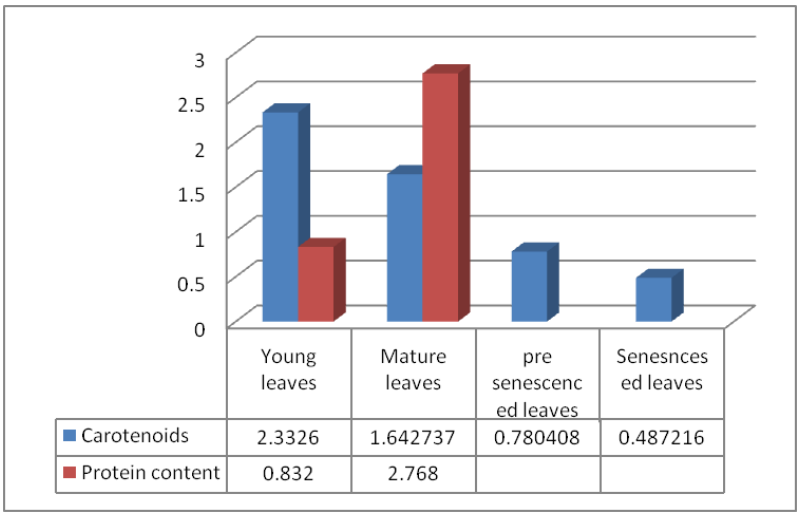

Fig. 3. Total Carotenoid Content and total protein content

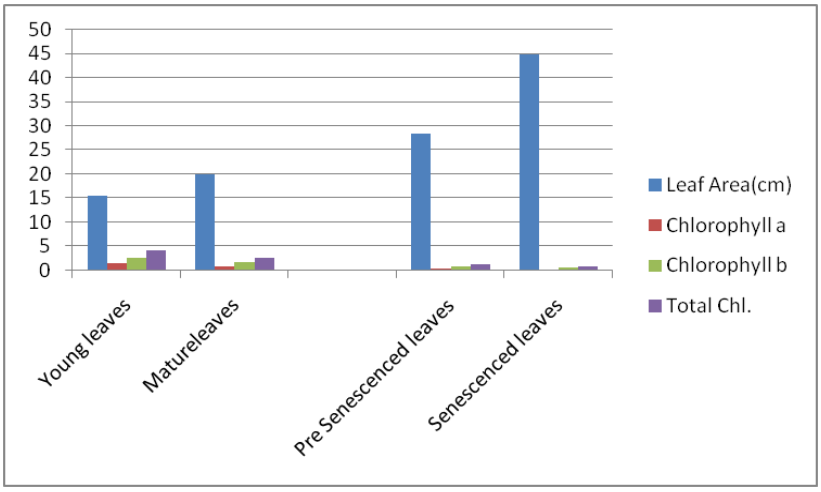

Fig. 4. Total chlorophyll content in different parts of *Agathis robusta*

Young leaves had the highest reported carotenoid content, whereas mature leaves had the lowest. A class of phytochemicals known as carotenoids is what gives food its many hues. They are acknowledged as being crucial in preventing illnesses in people and preserving their health. Certain carotenoids are powerful antioxidants that also help the body produce vitamin A through food. The positive impact of phytochemicals in the prevention of several chronic illnesses is supported by scientific research. Carotenoids' chemistry has been thoroughly researched, but research on their bioavailability, metabolism, and biological roles is just now starting. The importance of lycopene to human health has drawn attention to carotenoids recently [17]. Highest Protein content observed in Mature Stem [18,19].

In above Fig. 4 maximum leaf area observed in Pre Senescenced leaves and Minimum in Young leaves. High Chlorolphyll a is observed in Young leaves and least observed in senescenced leaves. While, Chlorophyll b Highly observed in young leaves and less in senescenced and total chlorophyll respectively [20-24].

4. CONCLUSION

The mature leaf recorded the highest moisture percentage. Phytochemical screening is crucial for finding novel sources of chemicals that are significant for both medicine and industry. Therefore, extracts from these could be seen as a good source for useful drugs. Maximum flavonoid obsereved in mature leaf and minimum was retained by mature stem. The phenolic content is highest in mature leaves and lowest in mature stem. Mature stem of *Agathis Robusta* had the highest Reducing Sugar Content, while the mature leaf plant portion had the lowest. Carotenoid also found majorly in young leaves and least in senescenced leaves. Protein content is highly observed in mature leaves and least observed in young leaves. High chlorophyll a and chlorophyll b is observed in young leaves.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bailey, Frederick Manson.A Synopsis of the Queensland Flora. 1883;498.
2. Boland DJ, Brooker MIH, Chippendale GM, Hall N, Hyland BPM, Johnston RD, Kleinig DA, Jurner JD. Forest trees of Australia. Melbourne: Nelson, CSIRO; 1985.
3. Verma RS, Padalia RC, Goswami P, Verma SK, Chauhan A, Darokar MP. Chemical composition and antibacterial activity of the essential oil of Kauri Pine from India.J. Wood Chem. Technol. 2016;36:270–277.
4. Whitmore TC.*Agathis robusta* subsp. nesophila. Pl. Syst. Evol. 1980;135:64.
5. Dragota S, Riederer M. Comparative study on epicuticular leaf waxes of Araucaria araucana, Agathis robusta and Wollemia nobilis (Araucariaceae). Australian Journal of Botany. 2008;56(8):644-650.
6. Brophy JJ, Goldsack RJ, Wu MZ, Fookes CJ, Forster PI. The steam volatile oil of Wollemia nobilis and its comparison with other members of the Araucariaceae (Agathis and Araucaria). Biochemical Systematics and Ecology. 2000;28(6):563-578.
7. Bisht B, Nainwal P, Saini P. Evaluation of *In vitro* anti-inflammatory activity of Agathis robusta. J. Pharma. Res. 2012;2:1304-1306.
8. Nielsen SS. Determination of Moisture Content. In: Nielsen SS. (eds) Food analysis laboratory manual. Food Science Texts Series. Springer, Boston, MA; 2010. Avaliable:https://doi.org/10.1007/978-1-4419-1463-7_3
9. Bray HG, Thorpe WV. Analysis of phenolic compounds of interest in metabolism. Methods of biochemical analysis. 1954;1.
10. Jensen A. Chlorophylls and carotenoides in Handbook of Phycological Methods, Physiological and Biochemical Methods; 1978.
11. Bradford MM. A rapid and sensitive method for the estimation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 1976;72:248–254.
12. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis. 2002;10(3).

13. Singh R, Singh SK, Arora S. Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auriculiformis* A. Cunn. Fod Chem. Toxicol. 2007;45:1216-1223.
14. Han X, Shen T, Lou H. Dietry polyphenols and their biological significance. Int. J. Mol. Sci. 2007;950-988.
15. Del-Rio A, Obdululio BG, Casfillo J, Main FG, Ortuno A. Uses and properties of citrus flavonoids. J. Agric. Food Chem. 1997;45:4505-4515.
16. Rolland F, Moore B, Sheen J. Sugar sensing and signaling in plants, The Plant Cell. 14, no. Supplement. 2002;1:S185–S205.
Available:https://doi.org/10.1105/tpc.010455,12045277
17. Rao AV, Rao LG. Carotenoids and human health, Pharmacological Research; 2007. ISSN 1043-6618,
Available:https://doi.org/10.1016/j.phrs.2007.01.012
18. Akindele AJ, Adeyemi OO. Anti-inflammatory activity of the aqueous leaf extract of *Byrosocarpus coccineus*. Fitoterapia. 2007;78:25-28.
19. Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahuand A, Bora U. Indian medicinal herbs as source of antioxidants. Food Res. Int. 2008;41:1-15.
20. Moore ex C, Muell F, Quart J. *Dammara robusta* Trans. Pharm. Soc. Victoria 2: 174.In: Quart. J. Trans. Pharm. Soc. Victoria. 1860;2:173.
21. Krings U, Berger RG. Antioxidant activity of roasted foods. Food Chem. 2001;72:223-229.
22. Polyphenolic flavonoids as scavenger of aqueous phase radicals as chai breaking antioxidant. Arc. Biochem. Broph. 2001;2: 339-346.
23. Chen Z, He D, Deng J, Zhu J, Mao Q. Chemical composition and antibacterial activity of the essential oil from *Agathis dammara* (Lamb.) Rich fresh leaves. Natural Product Research. 2015;29(21):2050-2053.
24. Dumbleton LJ. A new genus of seed-infesting micropterygid moths; 1952.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/120130