ORIGINAL ARTICLE

Isolation and characterization of catechol derivatives from *Semecarpus anacardium* nuts

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Abstract

Aim: The aim of this study is to isolate the compounds from the methanolic extract of *Semecarpus anacardium* seed. **Materials and Methods:** Thin layer and column chromatography techniques have been used for the isolation of compounds and their structural confirmations were made based on spectral data. **Results and Discussion:** The four purified fractions were subjected to infrared, ^{1}H NMR, ^{13}C NMR, and high-resolution mass spectrometry spectral analysis for structural confirmation. From the spectral data, these four fractions were confirmed as catechol derivatives. **Conclusion:** From the obtained spectral data, the fractions were confirmed as catechol derivatives.

Key words: Catechol derivatives, high-resolution mass spectrometry, infrared, NMR, *Semecarpus anacardium*

INTRODUCTION

Traditional use of plant origin remedies for the treatment of diseases is widely practiced in both developed and developing countries. It is estimated that approximately 60% of the world's population relies on plants for medications. This percentage raise to >80% due to the expansion of populations in the developing world, easy access, and escalating drug costs.[1,2] Therefore, plants remain the major supplier of active drugs from natural sources.[3] Plants contain pharmacologically active components which are relatively safe and frequently considered to be less toxic and free from side effects than synthetic ones.[4] Plants derived components play a key role in world health and have long been known to possess biological activity.[5] Thirty percent of all modern drugs are derived from plants.[6] According to the World Health Organization, about 80% of the world's population living in developing countries relies essentially on plants for primary health care.[7-9]

The chemical constituents of plants are desirable to understand herbal drugs and their preparations. Most importantly, these studies will be helpful to isolate and characterize the chemical constituents present in those plant extracts. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies. *Semecarpus anacardium* belongs to the family *Anacardiaceae*, grown in sub-Himalayan, tropical, and central parts of India. Seeds of *S. anacardium* have been used in the Indian traditional system of Medicines (Ayurveda and Siddha) either alone or as an ingredient of many polyherbal formulations for treating various ailments. Ayurveda describes the *S. anacardium* as a potent drug for arthritis, leprosy helmintic infection, and venereal disorders.[10-12] The nut milk extract of *S. anacardium is* one of the formulations of Siddha Medicine in India, and this preparation is called as "Serankottai nei." Cow's milk and ghee are the main ingredients of this drug and its pharmacological effects have

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been proved in our laboratories such as anti-oxidant, anti-inflammatory, anti-arthritis, anti-cancer, and anti-diabetic.[13-17] Apart from our investigation, some other investigators have also been reported that various extracts of *S. anacardium* nuts such as aqueous and alcoholic extracts possessed to have anti-microbial and anti-bacterial activities.[18,19] However, the mechanism of the pharmacological action of its nuts can be greatly aided by the isolation of its active principles and the determination of structure and functional relationship. These informations encouraged us to isolate the active principle from *S. anacardium* nuts. *S. anacardium* seeds have already been subjected to broad investigations and isolation of a number of compounds including tetrahydroamentoflavone[20] semicarpoflavonone[21] jeediflavanone,[22] galluflavonone,[23] nallaflavonone[24] semecarpetin,[25] anacardioflavonone, etc.[26] Moreover, there are a number of catechol derivatives have been reported in some other *Semecarpus* species.[27] Based on the wide pharmacological activities of *S. anacardium*, we planned to isolate the compounds from the methanolic extract of *S. anacardium* seeds.

MATERIALS AND METHODS

General Experimental Procedures

Ultraviolet (UV) spectra were recorded with a UV160A–Simadzu spectrophotometer. The infrared (IR) spectra were recorded with a Thermo Satellite Fourier Transform-IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded using 300 and 75.1 MHz Bruker spectrometer with $CDCl_3$ (deuterated chloroform) as solvent, and chemical shifts are recorded in parts per million with tetramethylsilane as an internal reference. The mass spectrum was obtained from time-of-flight high-resolution mass spectrometry (TOFHRMS) mass spectrometers. Column chromatography was performed on silica gel 60–120 mesh (Merck). Precoated plates of silica gel 60 F_{254} were used for analytical purposes.

Plant Material

S. anacardium seeds were purchased from K. R. Vasan Traditional and Herbal Medicine shop, Parris, Chennai, Tamil Nadu, India. The identity of the plant was confirmed by Prof. Raman, plant taxonomist, Centre for Advanced Studies in Botany, University of Madras. The voucher specimen (MUCASB- H105) was deposited in the department herbarium.

Extraction and Isolation

A total of 1000 g of *S. anacardium seeds* were bruised and soaked in 2.5 l of methanol and kept in the refrigerator for 3 days. Then, the filtrate was filtered through Whatman filter paper No. 1, and this was repeated 3–4 times until the filtrate gave no coloration and concentrated using vacuum rotary evaporator at 40°C. The methanolic concentrate was fractionated sequentially with petroleum, diethyl ether, chloroform, and n-butanol. The n- butanolic fraction was evaporated to dryness, and n-butanolic concentrate was checked on thin-layer chromatography (TLC) with hexane and ethyl acetate in the ratio of 8:2 which showed four spots (compounds). The n-butanolic concentrate was chromatographed on silica gel column (Merk 60–120 mesh, 750 g, 3.5 i.d. × 60 cm) and eluted with hexane and ethyl acetate (80:20 ratio). A total of 50 fractions were collected at an interval of 10 ml each and monitored by TLC (precoated silica gel merk-60 F_{254} 0.25 mm thick plate). Fractions from 1 to 5 formed as a pale green or straw yellow color and showed single spot on TLC and pooled together in a clean vial and evaporated to dryness and yield of the compound was 1.8 g/kg and colloidal in nature. Fractions from 15-20 were dark yellow colour and showed single spot on TLC and pooled together in a clean vial and evaporated. The yield of the compound was 1.00 g/kg. The physical nature of the compound was colloidal in form. Fraction from 25 to 30 formed as light yellow color and showed single spot on TLC and pooled together in a clean vial and evaporated to dryness and yield of the compound was 0.80 g/kg. Fraction from 40 to 50 formed as deep red color and showed single spot on TLC and pooled together in a clean vial and evaporated to dryness to yield 2.4 g/kg. The nature of the compound was colloidal in form. This process was repeated until getting satisfactory yield of each compound. The structure of the compound was confirmed as catechol derivatives on the basis of IR,^{1}H NMR,^{13}C NMR, and high-resolution mass spectrometry (HRMS) spectral data.

RESULTS AND DISCUSSION

Spectroscopic Description of Catechol Derivative I [Figure 1]

The compound I was obtained as a straw yellow color and colloidal in nature. The molecular formula of this compound was determined from IR, TOFHRMS, ^{1}H and ^{13}C NMR. In the IR spectrum, a peak at 3455.81 cm^{-1} confirmed the presence of –OH group in the compound [Figure 2]. In the ^{1}H NMR spectrum a broad singlet peak at δ 5.26 ppm corresponds to the -OH group present in the compound. The aromatic protons resonated as an apparent singlet at δ 6.55 ppm. The aliphatic protons present in the compound appeared at δ 0.80–2.49 ppm. The methyl group present in the aromatic

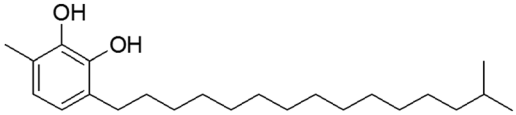

Figure 1: Chemical structure of catechol derivative I

ring resonated as a singlet at δ 2.68 ppm [Figure 3]. In the ^{13}C NMR spectrum, the two methyl groups present in the compound observed at δ 13.88 ppm and δ 14.21 ppm. The aromatic protons appeared at δ 120.32, 122.2, 128.08, 129.98, 142.05, and 143.19. All the other aliphatic carbons observed at δ 22.78–29.98 ppm. The methyl group present in the aromatic ring appeared at δ 31.90 ppm [Figure 4]. Finally, the structure of catechol derivative I was confirmed by HRMS (EI) calcd for $C_{23}H_{40}O_2$ (M+Na): 371.2926. Found 371.2935 [Figure 5]. IR 3455.81 (-OH) cm^{-1},^{1}H NMR (300 MHz $CDCl_3$) δ 0.808 (s, 3H, CH_3), 0.832 (s, 3H, CH_3), 1.193 (s, 22H, CH_2), 1.948-1.929 (m, 1H), 2.440-2.490 (m, 2H), 2.681(s, 3H, CH_3), 5.263 (s, 2H, OH), 6.558 (s, 2H Ar-H) ppm.^{13}C NMR (75.1 MHz, $CDCl_3$) δ 13.88, 14.21, 22.78, 22.89, 25.75, 27.33 (CH), 29.10, 29.39, 29.57, 29.64, 29.78, 29.85, 31.90, 39.18, 120.32 (Ar), 122.20 (Ar), 128.08 (Ar-CH_3), 129.98 (Ar), 142.05 (Ar-OH), 143.19 (Ar-OH) ppm. HRMS (EI) calcd for $C_{23}H_{40}O_2$ (M+Na): 371.2926. Found 371.2935.

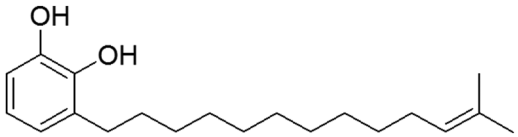

Figure 2: Chemical structure of catechol derivative II

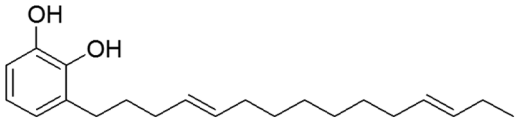

Figure 3: Chemical structure of catechol derivative III

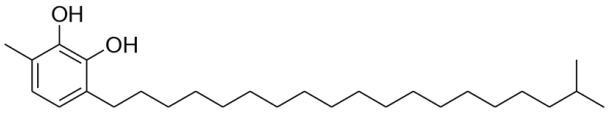

Figure 4: Chemical structure of catechol derivative IV

Spectroscopic Description of Catechol Derivative II [Figure 6]

The compound 2 was obtained as a dark yellow color, colloidal in form. The molecular formula of this compound was determined on the basis of IR, TOFHRMS, ^{1}H and ^{13}C NMR. In the ^{1}H NMR spectrum of the compound, a peak was observed at δ 5.35 ppm, which confirmed two –OH groups present in the compound [Figure 7]. The aromatic protons resonated as multiplets in the region δ 6.65–6.73 ppm. The aliphatic protons present in the compound resonated at δ 0.88–2.64 ppm [Figure 8]. In the^{13}C NMR spectrum, the one methyl protons present in the compound appeared at δ 14.13 ppm. The aromatic protons appeared at δ 121.61, 122.37, and 128.82. The other aliphatic protons appeared at δ 22.82, 25.68, 27.26, 29.02, 29.32, 29.45, 29.50, 29.62, 29.73, 29.83, and 31.82 ppm [Figure 9]. Finally, the structure of catechol derivative II was confirmed by HRMS (EI) calcd for $C_{20}H_{32}O_2$ (M+K): 343.2039. Found 343.2045 [Figure 10] IR 3467.13 (-OH) cm^{-1}, ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.889 (d, 6H, J = 5.7Hz), 1.259 (br, s, 14H, CH_2), 1.60 (br, S, 2H, CH_2), 2.023 (br, s, 2H, CH_2), 2.579–2.645 (m, 2H, CH_2), 5.350 (s, 2H, OH), 5.632 (br, s, 1H, CH), 6.650–6.730 (m, 3H, Ar-H) ppm. ^{13}C NMR (75.1MHz, $CDCl_3$) δ 14.13 (CH_3), 22.82 (CH_3), 28.82 (CH_2), 27.26, 29.02, 29.32, 29.45, 29.50, 29.62, 29.73, 29.83, 31.82, 113.32 (Ar-CH) 121.61 (Ar-CH), 122.37 (Ar-CH), 125.79 (CH), 128.82 (Ar-CH), 130.39 (CH), 142.13 (Ar-OH), 143.54 (Ar -OH) ppm. HRMS (EI) calcd for $C_{20}H_{32}O_2$ (M+K): 343.2039. Found 343.2045.

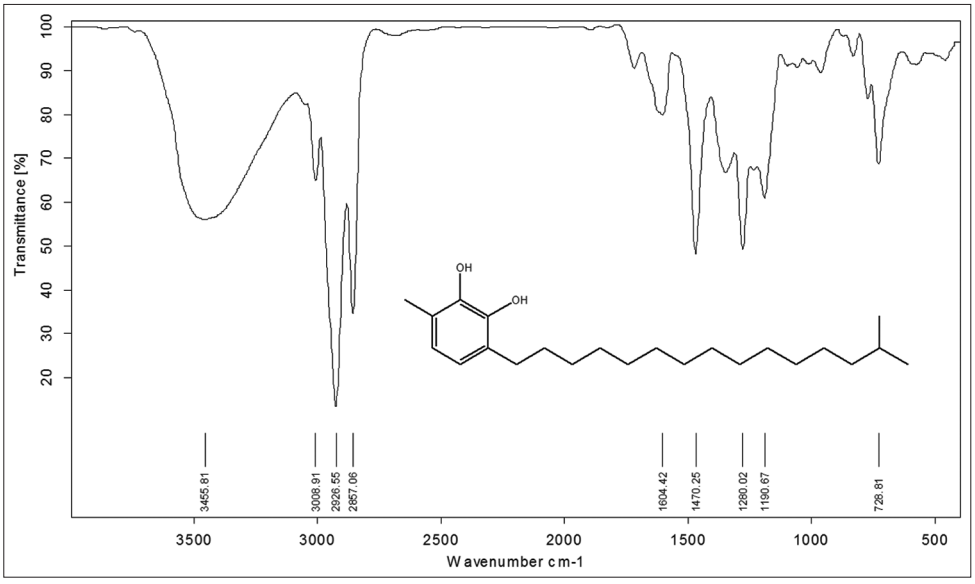

Figure 5: Scanned copy of IR spectra of catechol derivative I

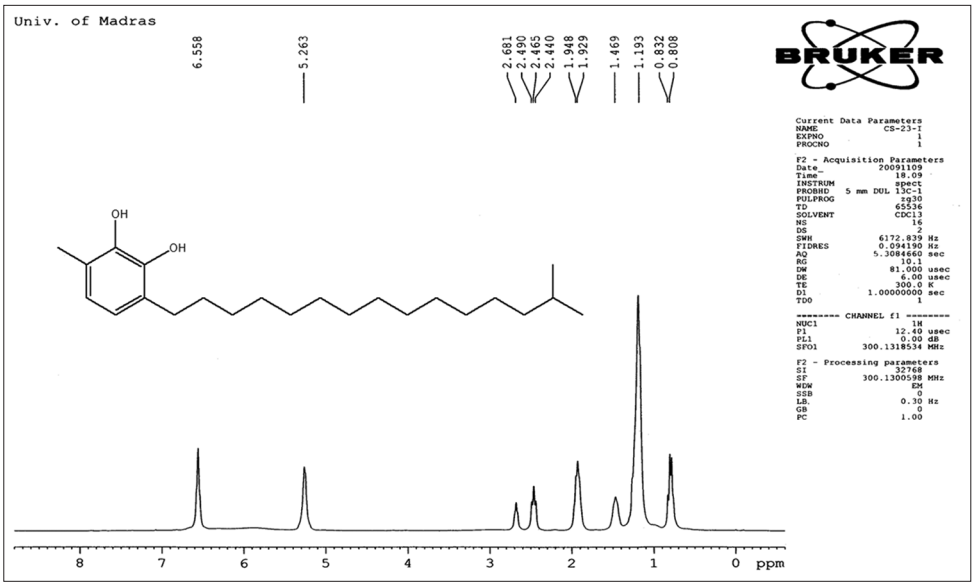

Figure 6: Scanned copy of ^{1}H NMR of catechol derivative I

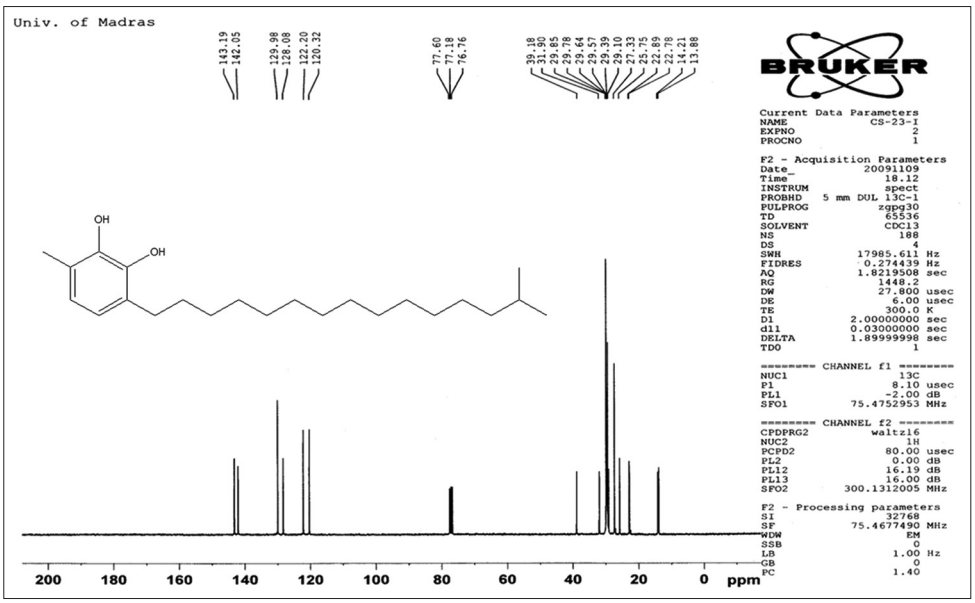

Figure 7: Scanned copy of ^{13}C NMR of catechol derivative I

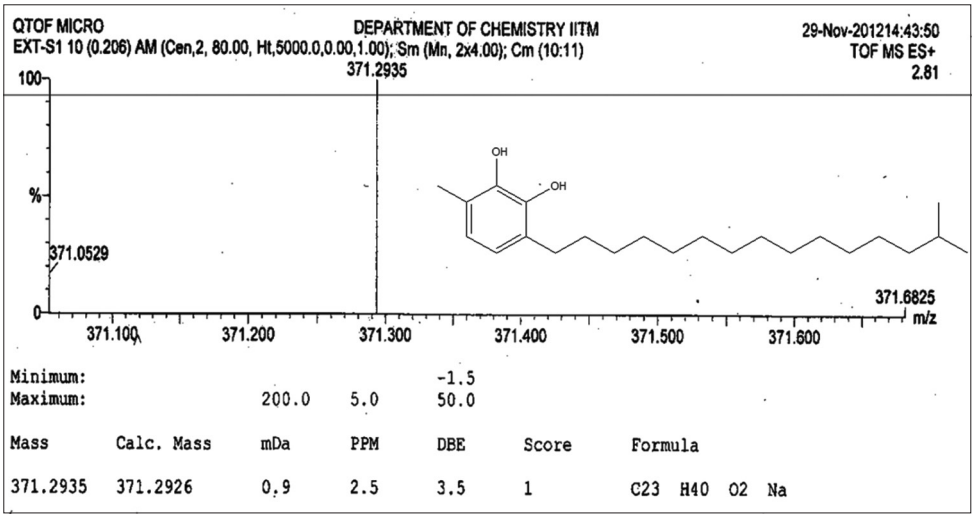

Figure 8: Scanned copy of HRMS of catechol derivative I

SPECTROSCOPIC DESCRIPTION OF CATECHOL DERIVATIVE III [FIGURE 11]

The compound 3 was obtained as light yellow color and viscous in nature. In the ^{1}H NMR spectrums, the compound exhibited a broad singlet at δ 5.35 ppm corresponding to the two –OH protons present in the compound [Figure 12]. The aromatic protons resonated as an apparent multiplet at δ 6.49–6.68 ppm. The aliphatic chain attached to the catechol ring was observed as multiplet at δ 0.85–2.77 ppm [Figure 13]. In the ^{13}C NMR spectrum, the carbons of the aromatic ring were observed at 112.95, 120.05, 121.07, 122.06, 142.20, 143.16, and aliphatic alkenes chain 127.9, 128.1, 129.9, and 130.2 ppm. All the other aliphatic carbons resonated at 22.67–31.9 ppm [Figure 14]. Finally, the structure of catechol derivative was confirmed by HRMS (EI) calcd for $C_{21}H_{32}O_2$ (M+H): 317.2481 Found 317.2482 [Figure 15]. IR 3404.75 (-OH) cm^{-1},^{1}H'NMR (300 MH_Z, $CDCl_3$), δ 0.879 (t, 3H, J = 6.75 Hz) 1.256–1.386 (br, s, 8H, CH_2), 2.018 (br, s, 2H, CH_2), 2.557–2.636 (m, 8H, CH_2), 2.772 (s, 2H, CH_2) 5.354 (s, 2H, Ar-OH), 5.639 (br, s, 4H, Aliphatic alkene), 6.489–6.685 (m, 3H, Ar-H) ppm.^{13}C NMR (75.1 MHz, $CDCl_3$) δ 14.11 (CH_3), 22.67, 22.81, 25.66, 27.24, 29.30, 29.37, 29.49, 29.71, 31.80, 31.94, 112.95 (Ar), 120.05 (Ar), 121.07 (Ar), 122.06 (Ar), 127.98 (Aliphatic Alkene), 129.46 (Aliphatic Alkene), 129.93 (Aliphatic Alkene), 130.20 (Aliphatic Alkene),

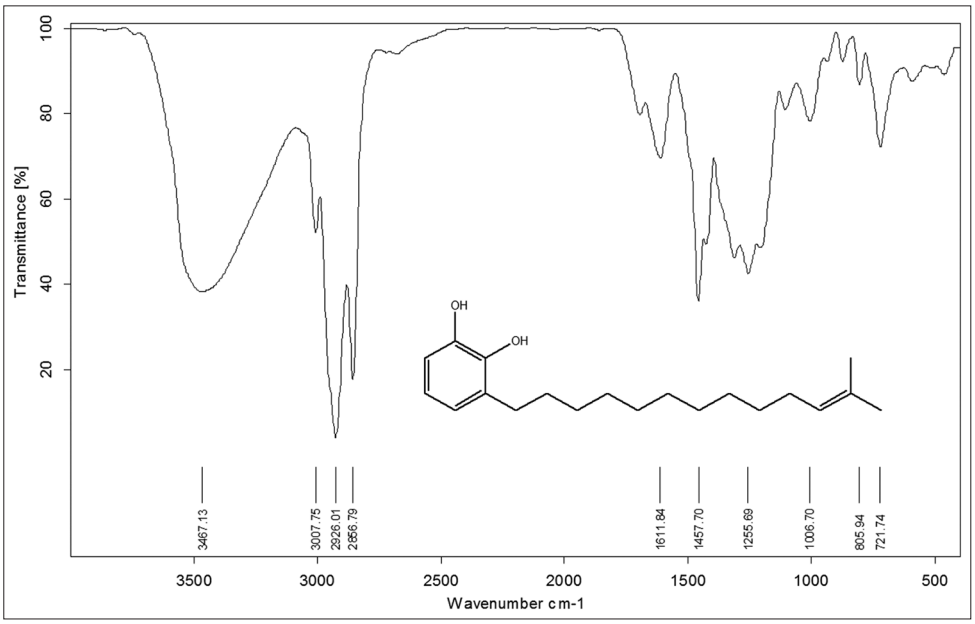

Figure 9: Scanned copy of IR spectra of catechol derivative II

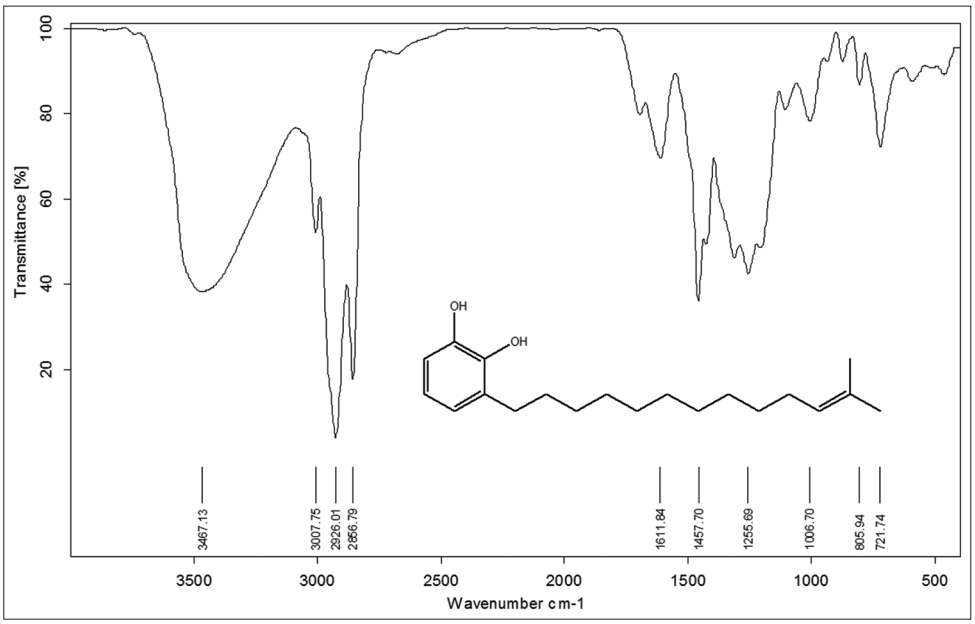

Figure 10: Scanned copy of 1H NMR spectra of catechol derivative II

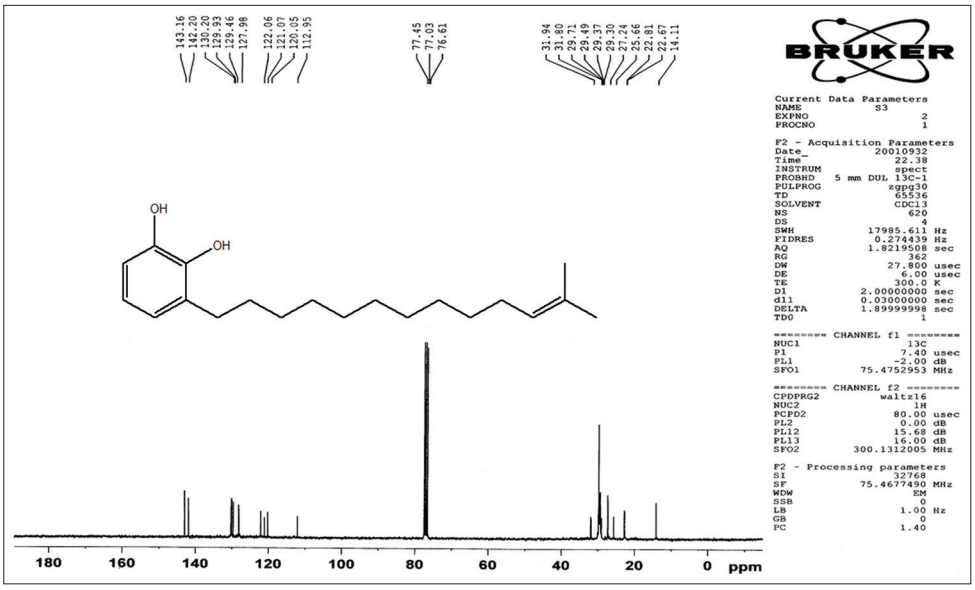

Figure 11: Scanned copy of ^{13}C NMR spectra of catechol derivative II

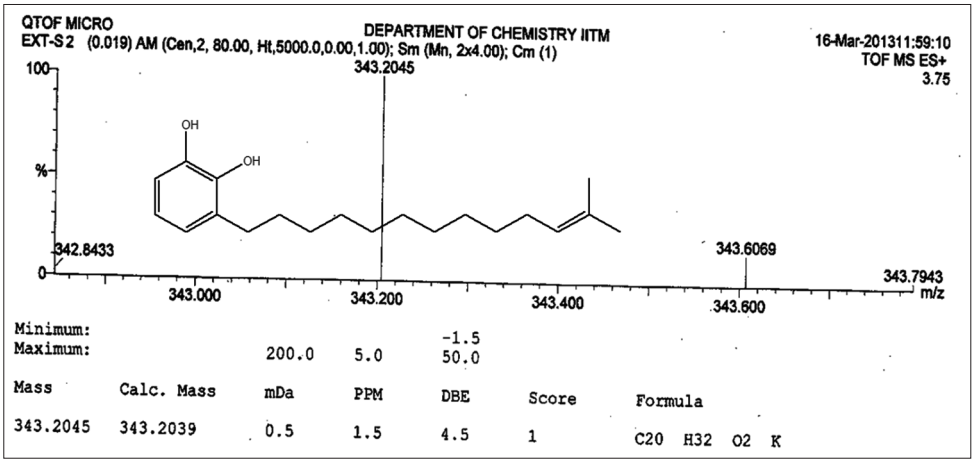

Figure 12: Scanned copy of HRMS of catechol derivative II

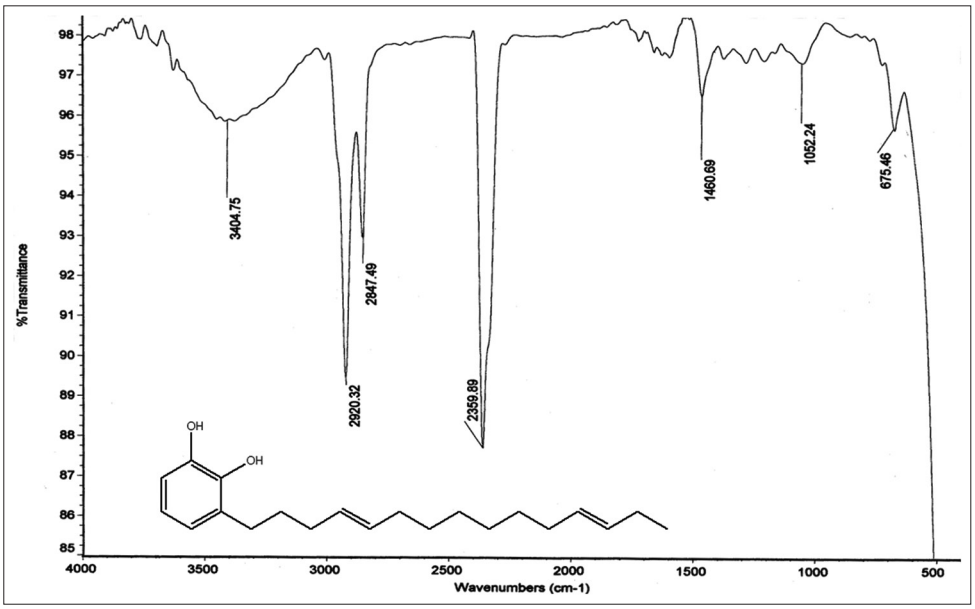

Figure 13: Scanned copy of IR spectra of catechol derivative III

142.20 (Ar-OH), 143.16 (Ar-OH) ppm. HRMS (EI) calcd for $C_{21}H_{32}O_2$ (M+H): 317.2481 Found 317.2482.

Spectroscopic Description of Catechol Derivative IV [Figure 16]

The compound 4 was obtained as a deep red in color and colloidal in nature. In the IR spectrum of the compound, a peak was observed at 3421.89 cm^{-1} which indicate the presence of –OH group present in the compound. In the ^1H NMR spectrum, the compound exhibited a broad singlet at δ 5.348 correspondings to the two–OH protons present in the compound [Figure 17]. The aromatic protons were observed at δ 6.68–6.82 ppm. The aliphatic protons attached to the catechol ring were observed as a multiplet at δ 0.91–δ 1.99. The methyl group present in the aromatic ring was observed at δ 2.60 ppm [Figure 18]. In the ^{13}C NMR spectrum, the aliphatic groups resonated at δ 14.13 ppm. All the other aliphatic carbons were observed at δ 22.68–39.60 ppm. A peak at δ 31.95 ppm corresponding to the methyl group was present in the aromatic ring. The aromatic carbons resonated at δ 128.0, 128.1, 129.6, 129.9, 141.2, and 143.2 ppm [Figure 19]. Finally, the structure of catechol derivative was confirmed by HRMS (EI) calcd for $C_{27}H_{48}O_2$ (M+Na): 427.3552 Found 427.3566 [Figure 20] IR 3421.89 (-OH) cm^{-1},^1H NMR (300 MHz, $CDCl_3$) δ 0.91 (d, 6H, J = 7.2 Hz), 1.255-1.305 (m, 30H, CH_2) 1.603 (s, 2H, CH_2), 1.996 (s, 1H, CH), 2.606 (S, 3H, CH_3), 2.751-2.786 (m, 2H, CH_2), 5.348 (s, 2H, OH), and 6.681-6.827 (m, 2H, Ar-H) ppm.^{13}C NMR (75.1MHz, $CDCl_3$) δ 14.13 (CH_3), 22.68, 22.71, 22.81, 25.66, 27.24, 29.00, 29.30, 29.33, 29.50,

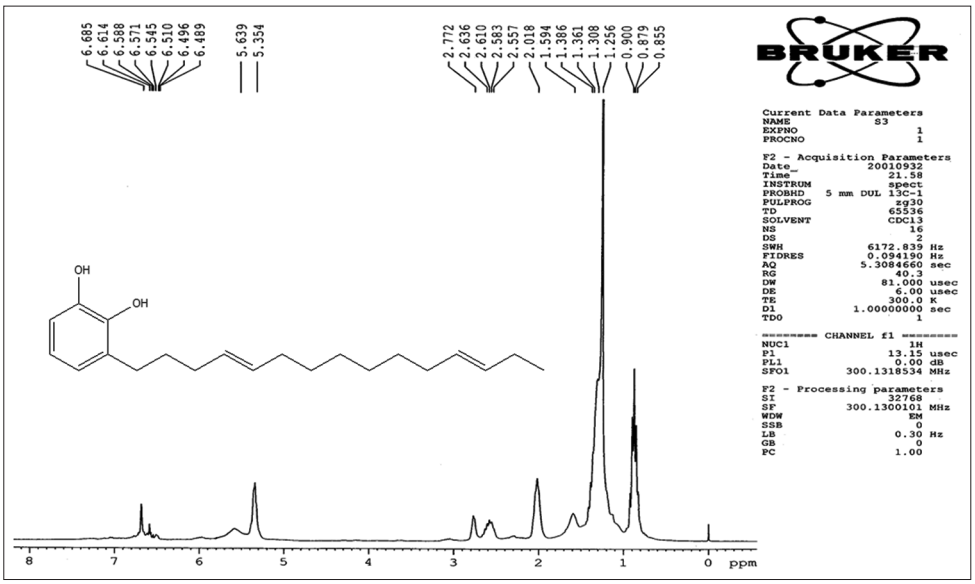

Figure 14: Scanned copy of ^1H NMR spectra of catechol derivative III

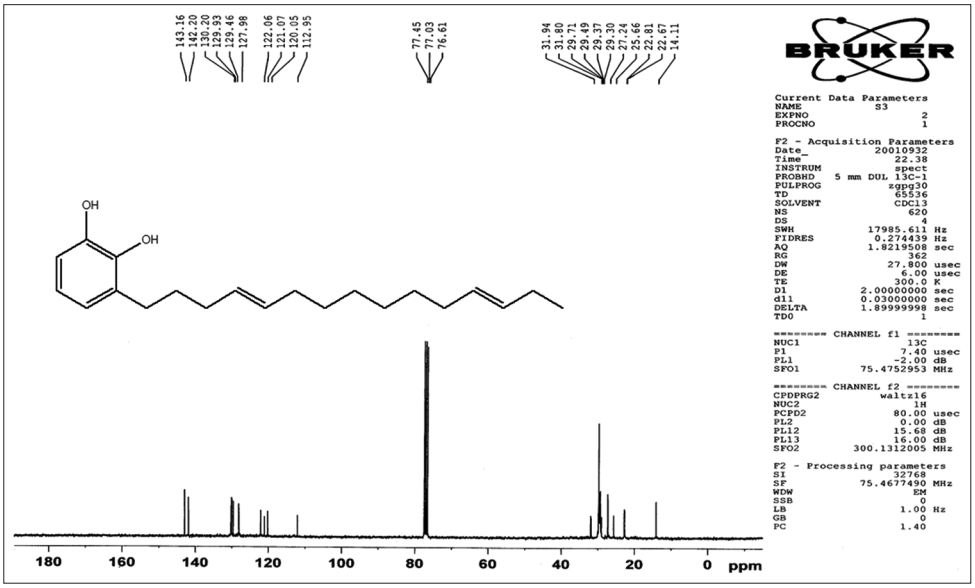

Figure 15: Scanned copy of ^{13}C NMR spectra of catechol derivative III

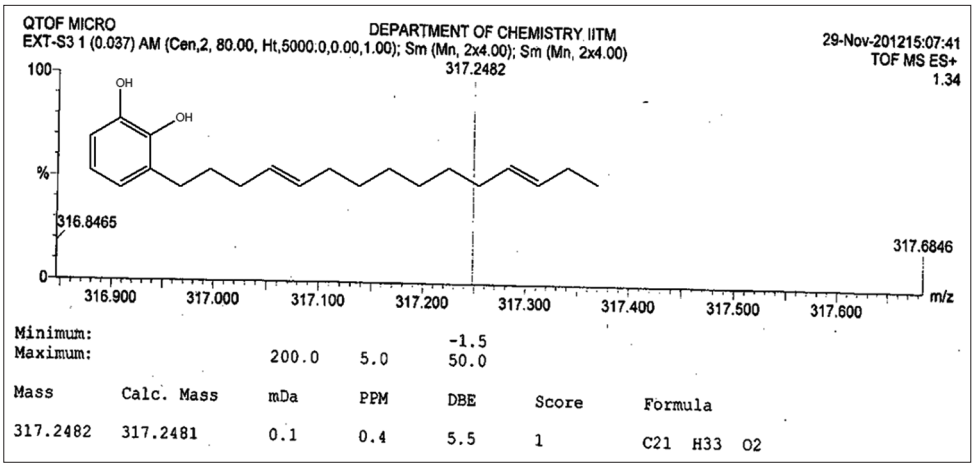

Figure 16: Scanned copy of HRMS spectra of catechol derivative III

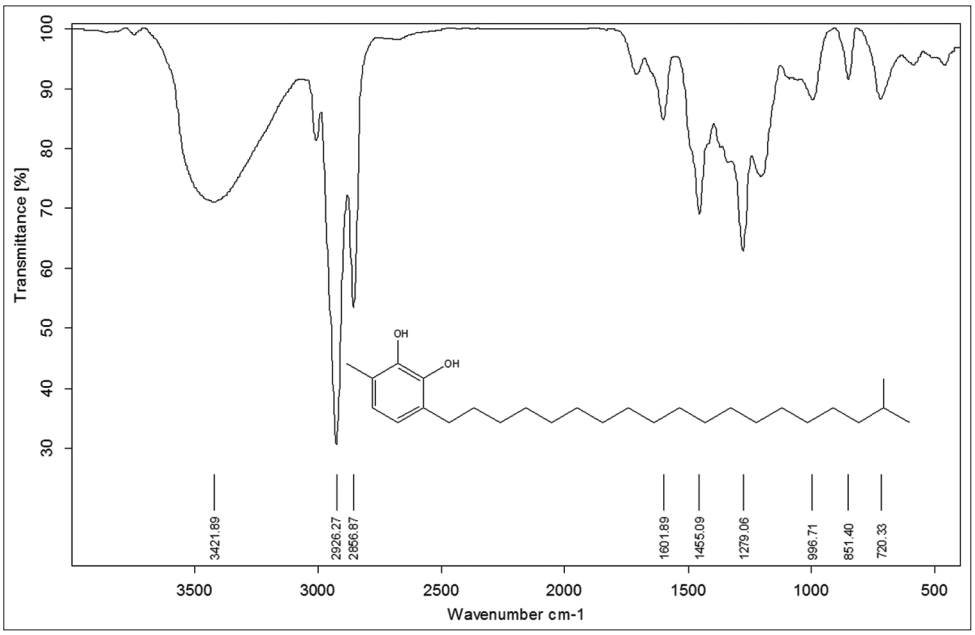

Figure 17: Scanned copy of IR spectra of catechol derivative IV

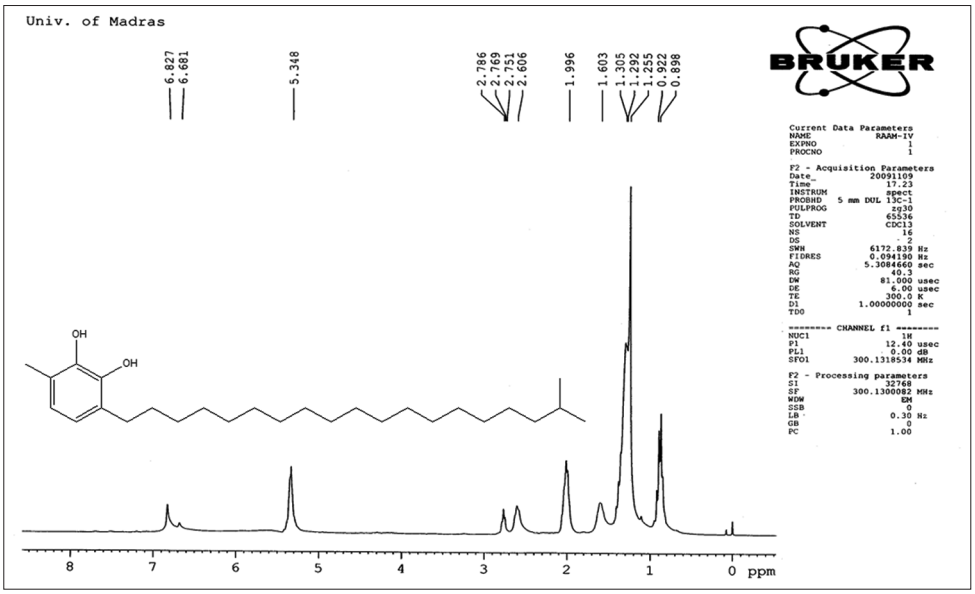

Figure 18: Scanned copy of ^{1}H NMR spectra of catechol derivative IV

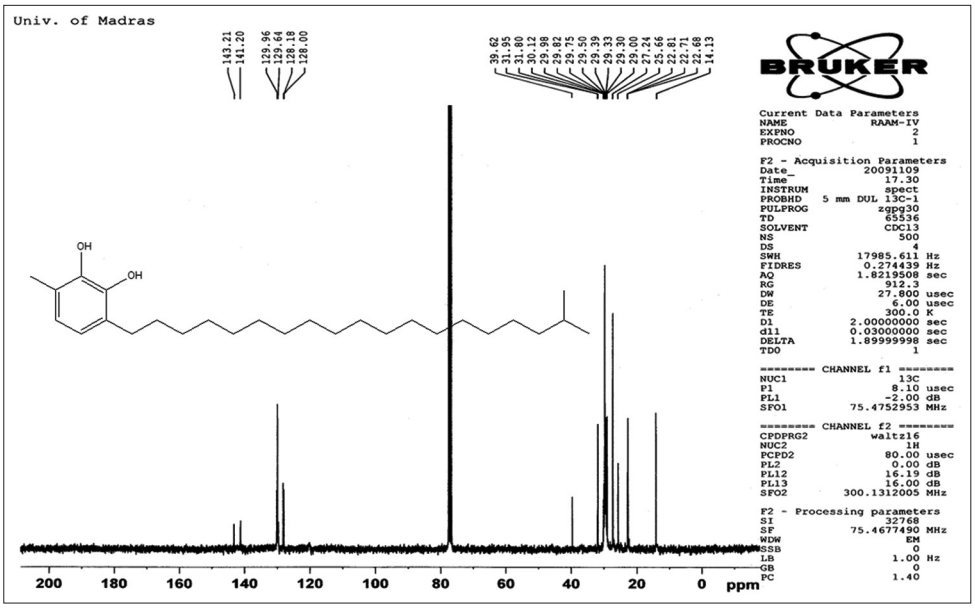

Figure 19: Scanned copy of ^{13}C NMR spectra of catechol derivative IV

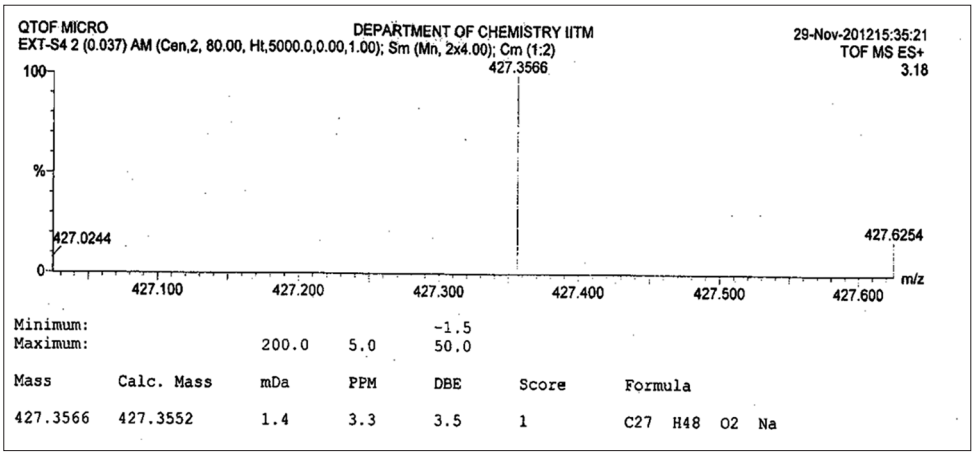

Figure 20: Scanned copy of HRMS spectra of catechol derivative IV

29.75, 29.82, 29.98, 30.12, 31.80, 31.95, 39.62, 128.00 (Ar-CH), 128.18 (Ar-CH), 129.64 (Ar-CH), 129.96, 141.20 (Ar-OH), 143.21 (Ar-OH) ppm. HRMS (EI) calcd for $C_{27}H_{48}O_2$ (M+Na): 427.3552 Found 427.3566.

CONCLUSION

Based on spectral data, these four compounds can be firmed as catechol derivatives.

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REFERENCES

1. Fransworth NR, Soejarto DD. Potencial consequence of plant extinction in the United States on the current and future availability of prescription drugs. Econ Bot 1994;39:231-40.
2. Cordell GA. Biodiversity and drug discovery a symbiotic relationship. Phytochemistry 2000;55:463-80.
3. Ogbonnia S, Adekunle AA, Bosa MK, Enwuru VN. Evaluation of acute and subacute toxicity of *Alstonia congensis* Engler *Apocynaceae*) bark and *Xylopia aethiopica* (Dunal) A. Rich (*Annonaceae*) fruits mixtures used in the treatment of diabetes Afr J Biotechnol 2008;7:701-5.
4. Gurib-Fakim A. Medicinal plants: Traditions of yesterday and drugs of tomorrow. Mol Aspects Med 2006;27:1-93.
5. Pribitkin EA. Herbal medicine and surgery. Semin Integr Med 2005;3:17-3.
6. Burns MM. Alternative medicine: Herbal preparation.

Clin Pediatr Emerg Med 2000;1:186-90.
7. McKay DL, Blumberg JB. A review of the bioactivity of South African herbal teas: Rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia intermedia*). Phytother Res 2007;21:1-6.
8. Fransworth N. The Role of Ethanopharmacology in Drug Development. Bioactive Compounds from Plant. New York: John Wiley and Sons; 2008.
9. Farnsworth NR. Biological and phytochemical screening of plants. J Pharm Sci 1966;55:225-76.
10. Chopra RN, Chopra IC, Handa KL, Kapur LD. In: Indigenous Drugs of India. 2nd ed. Calcutta: U.N. Dhur and Sons; 1958. p. 408.
11. Chattopadhyaya MK, Khare RL. Isolation of anacardic acid from *Semecarpus anacardium* Linn. And study of its antihelmintic activity. Indian J Pharmacol 1969;32:104-5.
12. Singh D, Aggarwal A, Mathias A, Naik S. Immunomodulatory activity of *Semecarpus anacardium* extract in mononuclear cells of normal individuals and rheumatoid arthritis patients. J Ethnopharmacol 2006;108:398-406.
13. Ramprasath VR, Shanthi P, Sachdanandam P. Evaluation of antioxidant effect of *Semecarpus anacardium* Linn. Nut extract on the components of immune system in adjuvant arthritis. Vascul Pharmacol 2005;42:179-86.
14. Ramprasath VR, Shanthi P, Sachdanandam P. Anti-inflammatory effect of *Semecarpus anacardium* Linn. Nut extract in acute and chronic inflammatory conditions. Biol Pharm Bull 2004;27:2028-31.
15. Ramprasath VR, Shanthi P, Sachdanandam P. Therapeutic effects of *Semecarpus anacardium* Linn. Nut milk extract on the changes associated with collagen and glycosaminoglycan metabolism in adjuvant arthritic Wistar rats. Chem Biol Interact 2006;162:43-52.
16. Mathivadhani P, Shanthi P, Sachdanandam P. Hypoxia and its downstream targets in DMBA induced mammary carcinoma: Protective role of *Semecarpus anacardium* nut extract. Chem Biol Interact 2007;167:31-40.
17. Aseervatham J, Palanivelu S, Sachdanandam P. Cytoprotective effect of *Semecarpus anacardium* against toxicity induced by streptozotocin in rats. J Exp Pharmacol 2010;2:135-43.
18. Mohanta TK, Patra JK, Rath SK, Pal DK, Thatoi, H.N. Evaluation of antimicrobial activity and phytochemical screening of oils and nuts of *Semecarpus anacardium.* Sci Res Essay 2007;2:486-90.
19. Nair A, Bhide SV. Antimicrobial properties of different parts of *Semecarpus anacardium*. Indian Drugs 1996;33:323-8.
20. Selvam C, Jachak SM. A cyclooxygenase (COX) inhibitory biflavonoid from the seeds of *Semecarpus anacardium*. J Ethnopharmacol 2004;95:209-12.
21. Murthy SS. A biflavanone from *Semecarpus anacardium.* Phytochemistry 1983;22:1518-20.
22. Murthy SS. Confirmation of the structure of jeedi flavonone: A biflavonane from *Semecarpus anacardium.* Phytochemistry 1984;3:925-7.
23. Murthy SS. Naturally occurring biflavonoid derivatives: Galluflavanone, a new biflavonoid from *Semecarpus anacardium*. Indian J Chem 1985;24b:398-402.
24. Murthy SS. New dimeric flavone from *Semecarpus anacardium*. Proc Indian Natl Sci Acad 1987;57:632-7.
25. Murthy SS. Semicarpetin: A biflavonoid from *Semecarpus anacardium.* Phytochemistry 1988;27:3020-2.
26. Murthy SS. New biflavonoid from *Semecarpus anacardium* Linn. Clin Acta Turcica 1992;20:30.
27. Carpenter C, Sotheeswaran S, Uvais M, Sultanbawa S. 5 methylmellein and catechol derivatives from four *Semecarpus s*pecies. Phytochemistry 1980;19:445-7.

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