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ORIGINAL ARTICLE **OPEN ACCESS**

5, 7, 4' Trihydroxyisoflavone Isolated From *Ipomoea Pes-Caprae* Roots by Normal Phase Column Chromatography

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ABSTRACT
It evaluates the result of active compound isolated from Ipomoea pes-caprae roots, gradually extracted by solvents and purified by silica gel column, resulted in a single compound has also been studied. The structure of isolated compound is characterized by using different spectrometric techniques like UV, IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry. The compound is 5, 7, 4' trihydroxyisoflavone (IUPAC name is 5, 7-Dihydroxy-3-(4-hydroxyphenyl) chromen-4-one) with molecular formula $C_{15}H_{10}O_5$ and molecular mass 270.05. EC_{50} value and in-vitro cytotoxic activities were studied for the purified compound with suitable assay method and results were discussed. The biological activity of the tested compound shows very effective anti-cancer activity against cell lines MCF-7. So it could be as good anti-cancer agent.
Keywords: *Ipomoea roots, TLC, silica gel, column, FT-IR, NMR, Mass.*

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INTRODUCTION

Plants are considered as effective and important anti-cancer agents. According to an estimate more than 60 % of recently utilized anti-cancer agents have originated from natural products. Microorganisms, marine organisms and plants are the major natural sources from which products of anticancer activity are obtained [1]. Alkaloids, such as vinblastine, vincristine, paclitaxel, docetaxel, camptothecin, colchicine, demecolcine are the compounds obtained from the plants that have ability to stop the cell division are used for the chemotherapy. Similarly other important sources of chemotherapy are the compounds derived from plants that have ability to kill the cancer cells, these compounds are known as cytotoxic compounds [2]. Antitumour activity derived from medicinal plants can be the result of a number of mechanisms. These mechanisms include the effects on cytoskeletal proteins that have important role in mitosis, inhibition of topoisomerase enzymic activity, activation of the immune system, or antioxidant activity [3]. Plant derived anticancer agents have great potential to be used as single agents or in combinational therapies. These agents have been used to treat localized or metastatic breast cancer. Paclitaxel is an example of anticancer compound first extracted from the *Taxus brevifolia.* It is complex diterpenoid compound [4]. Cancer is still a dreadful disease due to limited availability of effective drugs against cancer. Limitations linked with the present day chemotherapeutic agents to treat cancer are that they are highly expensive, mutagenic and sometimes carcinogenic. Their applications are therefore limited [5]. Therefore efforts are made to isolate and identify anticarcinogens that are naturally present in plants, which can effectively be used to prevent, slow or reverse cancer development. *Ipomoea pes-caprae* is one of the most widely distributed beach plants throughout tropical and subtropical areas in the world. It occurs along the beaches, coastal strands and tropical islands of tropical North and South America, east central Africa, west central Africa, India, Asia, and Australia. In North America, *I. pes-caprae* occurs from Florida, and west through the Gulf of Mexico and the Bahamas. Its range extends from approximately 30° North latitude to 30° South latitude. The extents of these limits are directly determined by climate, as *I. pes-caprae* does not tolerate prolonged frost conditions. Railroad vine occurs throughout the Indian River Lagoon on coastal dunes, scrub and some upland areas [6-9]. It has biological activity like anti-oxidant, analgesic and anti-inflammatory, antispasmodic, anti-cancer, antinociceptive, antihistaminic, insulogenic and hypoglycemic. It is also used in inhibition of platelet aggregation, diarrhoea, vomiting, and piles [10, 11]. The present investigation deals with the isolation of compounds

by using different chromatographic techniques from the most active extract of *I. pes-caprae* roots [12] and characterising the structure of isolated compounds by using different spectrometric methods.

MATERIAL AND METHODS

Thin Layer Chromatography (TLC)

TLC have been performed on a pre-coated silica gel TLC plates grade F_{254} (E-Merck, Darmstadt, Germany) to determine the number of compounds present in the given sample. The R_f values of the compounds were calculated using the following formula.

$$R_f = \frac{\text{distance travelled by the compound}}{\text{distance travelled by the solvent front}}$$

Column chromatography

Based upon the anti-microbial efficacy; the most active methanol extract of the sample of *I. pes-caprae* purified through Silica gel column chromatography. The concentrated crude metabolites were mixed with methanol–silica gel slurry and loaded into a silica gel 100–200 mesh (E-Merck, Darmstadt, Germany) column, packed in hexane: (the dimension of column was 450 × 30 mm). The column was eluted with stepwise gradient of chloroform/methanol (100:0; 90:10; 80:20; 70:30; 50:50; 30:70; 10:90, v/v) solvents. Each fraction have been concentrated and checked for its anti-oxidant activity. The fraction showing maximum anti-oxidant activity was further purified by silica gel 230–400 mesh (E-Merck, Darmstadt, Germany) column chromatography. The separation was done by gradient elution with low polar/high polar (gradient from 100 % low polar/0 % high polar to 0 % low polar/100 % high polar) using the flow rate of 2 ml/min. One hundred tubes of 10 ml each were collected and then analysed by TLC. Fractions showing similar spots with same R_f values were pooled and concentrated by a speed-vac under low pressure with evaporating temperature of 40°C. All the fractions were tested for their anti-oxidant activity by DPPH assay. The active compounds were checked for their purity by TLC.

Characterization of purified molecules

Physical properties: The physical appearance of the purified compounds was determined visually. Solubility was checked with methanol, ethyl acetate, chloroform, hexane, DMSO and water.

UV–vis spectra: The purified compounds were dissolved separately in methanol at 2–10 µg/ml concentrations and their UV–vis spectra were recorded using a UV-vis spectrophotometer (Shimadzu, Japan) between 200 and 800 nm. Methanol was used as blank.

FTIR: IR spectra for the purified compounds were recorded on a Perkin–Elmer 1600 series. FTIR spectrometer using KBr pellets.

NMR: ^{1}H NMR and ^{13}C NMR of the purified compounds were recorded in deuterated DMSO with tetramethylsilane (TMS) as internal standard solution using 400 MHz Bruker machine (Bruker, MA, USA).

Mass: The ESI-MS was recorded using the Thermo Finnigan LCQ Advantage MAX 6000 ESI mass spectrometer with nano-ESI-API-ion source (Finnigan, MAT, San Jose, CA). Isolated compounds were also subjected to biological activity.

RESULTS AND DISCUSSION

Isolation of Bio active compound from most active extract of I. pes-caprae roots

For isolation of the active principal, air-dried and finely ground roots of *Ipomoea pes- caprae* was extracted sequentially with solvents and most active methanol extract was assayed for anti-microbial efficacy. Most active methanol extract of *I. pes-caprae* roots were loaded to silica gel (100–200 mesh) column purification. The root crude sample of 5 g was fractioned through low resolution silica gel column chromatography using chloroform/methanol as eluting solvent. About 11 fractions were obtained and single spotted fractions on TLC were pooled together and concentrated for further studies (Fr.1–Fr.7). The schematic representation for the isolation of anti-oxidant compound is given. Among these, seventh fraction [Fr. 7] showed 61 % DPPH scavenging activity, while, Fr. 1 and 2 showed no activity while Fr. 3, 4, 5, and 6 showed 9.6 %, 27.4 %, 11 % and 31 % DPPH scavenging activity respectively. Further purification of the seventh fraction (Fr.7) by using high resolution silica gel (230–400 mesh) chromatography yielded about 10 fractions. The fractions having same number of spots with similar R_f values on TLC plate were pooled in sub six fractions Fr.7.1-7.6. Among these, third fraction [Fr. 7.3] showed 64. 8 % DPPH scavenging activity, while, Fr. 7.1, 7.2, 7.4, 7.5 and 7.6 showed 10.7 %, 24 %, 28.6 %, 32.5 % and 15 % DPPH scavenging activity respectively. Fr. 7.3 was further purified using high resolution column chromatography resulted 13 fractions, of which fractions 8-13 showed significant radical scavenging activity (72 % - 7.3.5) and a single spot on TLC. Among these, Fr. 7.3.1, Fr. 7.3.2, Fr.7.3.3 and Fr. 7.3.4 exhibited 17 %, 21 %, 46 % and 32 %. Physical characteristics of fractions of active compound were given, and they responded to specific tests for flavonoid compounds as recommended by

Stahl (1969) [13]. The isolated compound was subjected to various spectrometric methods viz. UV, IR, NMR and LC-MS to elucidate the structure.

Spectral analysis of the pure compound

Physical properties: A white amorphous powder compound with no UV absorbing band on TLC with the R_f value of 0.62 (9:1, chloroform: methanol) was purified from root part. This anti-bacterial compound was fully soluble in organic solvents such as methanol, ethanol and ethyl acetate. It is presented in figure 1. It was purified from most active methanol extracts of of *Ipomoea pes-caprae* roots by chromatographic techniques. TLC of compound shows single spot with good R_f values. It reveals the isolated compound was the pure compound without any mixtures. So, the isolated compound moved for further confirmation studies via UV, IR, ^{1}HNMR, ^{13}CNMR and mass spectrometric methods.

UV–vis spectrum: The spectrum of the purified compound solution in methanol exhibited absorption maximum at UV region 260 nm. It is presented in figure 2. Figure 2 shows the UV-Vis absorption spectrum of the compound, indicating this compound display maximum absorption in the vicinity of 260 nm. **FT-IR**: The IR spectrum of purified compound displayed an absorption band at ν_{max} 3432 cm^{-1} (hydroxyl group). The bands at ν_{max}1460 cm^{-1} are indicative for carbonyl carbon group. The IR spectrum of the isolated compound was shown in Figure 3. The IR spectra of compound show a broad band at 3432.81 cm^{-1} corresponding to the phenolic-OH group and carbonyl stretching vibrations in region 1641-1664 cm^{-1}. The FTIR spectrum has a sharp peak at 2925 cm^{-1}. Close to it is another medium narrow peak and it is formed at 2854 cm^{-1}. Both the peaks correspond to C-H steching frequency of alkyl groups. The carbonyl peak of the ketone group has shown absorption at longer wavelength. This is because of the conjugation with the C=C causing delocalisation of the electrons of C=O group and reducing the double bond character of carbon to oxygen bond, causing absorption at longer wavelength. O-C stretching band was seen at 1020.43 cm^{-1}. Aromatic C=C stretching bands were observed in the range of 1600-1400 cm^{-1}. The compound showed carbonyl stretching vibrations at 1641.42 cm^{-1}. **^{1}H NMR**: The ^{1}H NMR shift of the purified compound showed the aromatic proton appears as multiples in range δ 6.28 to 8.37. The hydroxyl OH proton appears as broad singlet δ12.28. ^{1}H NMR spectrum of isolated compound was shown in Figure 4. The ^{1}H NMR spectrum was recorded using DMSO-d6 as the solvent. Spectrum for purified compound was identical and showed the following details. The resonance peaks for aromatic protons and phenolic protons were found in the region of δ 6.0–8.5 and δ 9.0–13.0, respectively. In addition, most of the peaks were in the low field region except for peak at δ 2.5 for dimethyl sulfoxide (DMSO). The ^{1}HNMR (400 MHz, DMSO) spectrum showed : 12.28 (1H, s, 5- OH), 8.3 (1H, s, H-2), 7.7 (2H, d, J=3.4 HZ, H-2/ & H-6/), 6.8 (2H, d, J=3.4 HZ, H-3/ & H-5/), 6.4 (1H, d, J= 1.7 HZ, H-8), 6.2 (1H, d, J= 1.7 HZ, H-6). **^{13}C NMR:** The ^{13}C NMR spectrum showed the aromatic carbon appeared at aromatic region at δ 114.59, 115.35, 116.06, 117.73, 123.08, 123.24, 130.24, 133.27, 147.05, 150.28, 153.26, 157.43 ppm. The carbonyl carbon appears at δ 175.63 ppm. It is presented in figure 5. ^{13}C-NMR (100 MHz, DMSO) ppm: 175.63 (C-4), 157.63 (C-7), 153.26 (C-4/), 150.28 (C-5), 147.05 (C-9), 133.27 (C-2), 130.24 (C-2/ & C-6/), 123.24 (C-1/), 123.08 (C-3), 117.73 (C-3/ & C-5/), 116.06 (C-10), 115.35 (C-6), 114.59 (C-8). **Mass:** The ESI–MS spectrum of the purified compound of root part showed a molecular ion peak at m/z 271.53 (100% pure, M+) indicating the molecular mass of peak of the purified compound.

Full scan electro spray ionization (ESI) mass spectrum was performed. The mass scanning range (m/z) was from 100 to 400. The mass spectrum of the isolated sample has been depicted in Figure 6. The spectra of the isolated compound exactly match with the structure of the purified compound. It is observed from the spectra that the mass peak of 271 corresponding to M+1 is found to be genistein. Molecular weight of genistein is 270.24 g/mol. Mass spectrum showed the parent molecular ion at 270.24 g/mol. The molecular formula of the compound was found to be $C_{15}H_{10}O_5$. Thus it is confirmed that the isolated sample was genistein. The molecular weight search between 269 and 270 in the Novel Antibiotic database, PubChem, NIST and SDBS databases revealed that a most similar compound with molecular mass of 270.05. Based on the physico–chemical properties, the purified compound were identified as **5, 7,** 4'-trihydroxyisoflavone and its structure is depicted. Its molecular formula is $C_{15}H_{10}O_5$ and the IUPAC name is 5, 7-Dihydroxy-3-(4-hydroxyphenyl) chromen-4-one. Its calculated molecular weight is 270.24 g/mol and the exact mass is 270.24 g/mol**.** PubChem *CID:* 5280343, **Genistein.** It is presented in figure 7.

Bioactive properties of isolated compounds

EC_{50} Determination [14]

Effective concentration 50 was done by DPPH assay to determine DPPH radical scavenging activity of isolated compound. The results are presented in Figures 8. Genistein was isolated from most active methanol extracts of *I. pes-caprae* roots shows DPPH activity with an EC_{50} value is found to be 170 µg/ml. The anti-oxidant activity of this compound may be attributed to the presence of –OH and C=O groups, as reported in structurally similar compounds. It confirms the isolated compound could be a good anti-oxidant agent.

Anti-cancer activity [15]

The in-vitro anti-proliferative activities of this compound proved that cancer cell lines inhibited their proliferation significantly with the increase in drug concentration. It was observed that MCF-7 cell line more cytotoxicity effect was observed in drug in 24 hours treatment, it also revealed the increased concentration of drugs shown good toxicity over the cancer cell. Isolated compounds show more potent activity. The results are presented in Figures 9 and 10.

The result showed that the isolated compound exhibited a potent cytotoxic activity against the MCF-7 cell lines with IC50 value is found to be 300µg/ml. So, it could be a good anti-cancer agent.

Figure 1 TLC of Compound

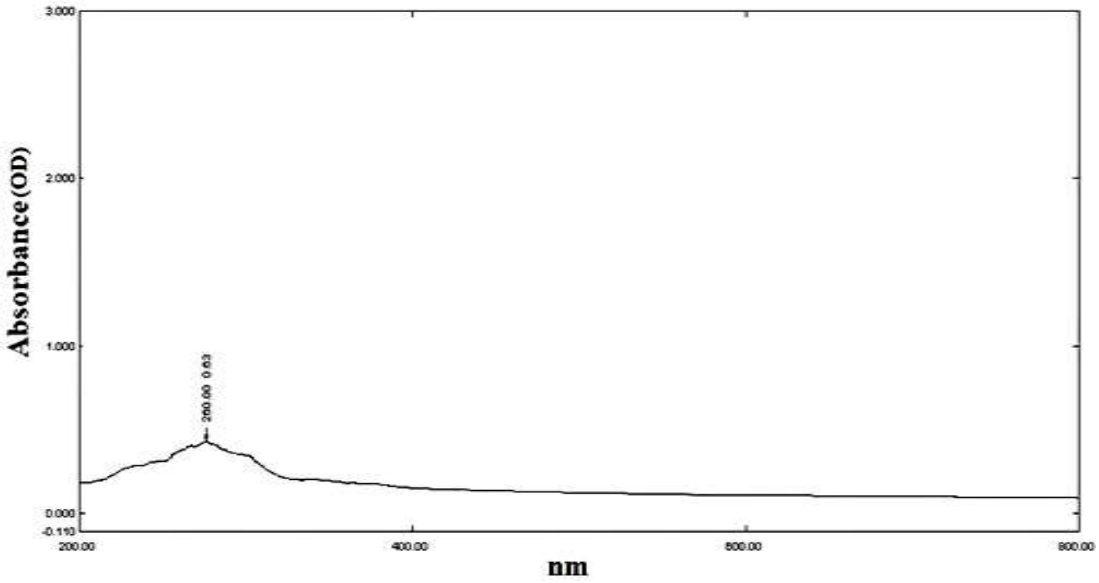


Figure 2 UV-vis spectrum of Compound

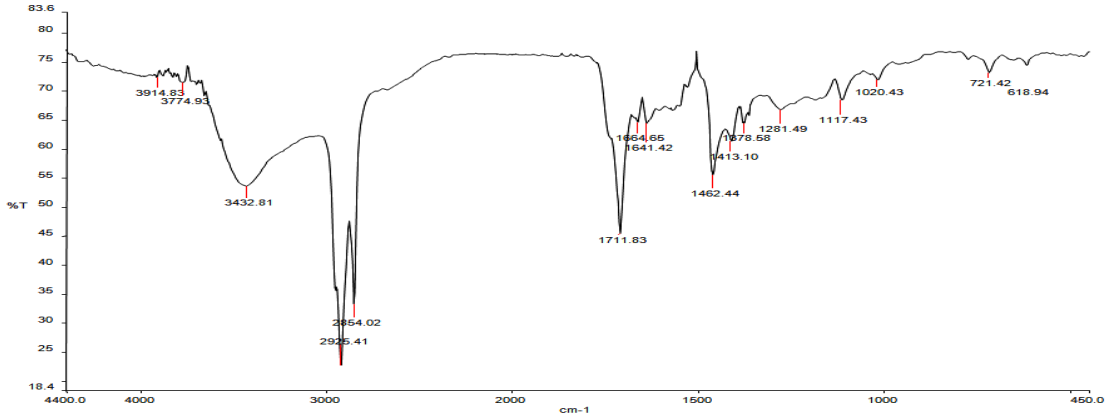


Figure 3 FT-IR spectrum of Compound

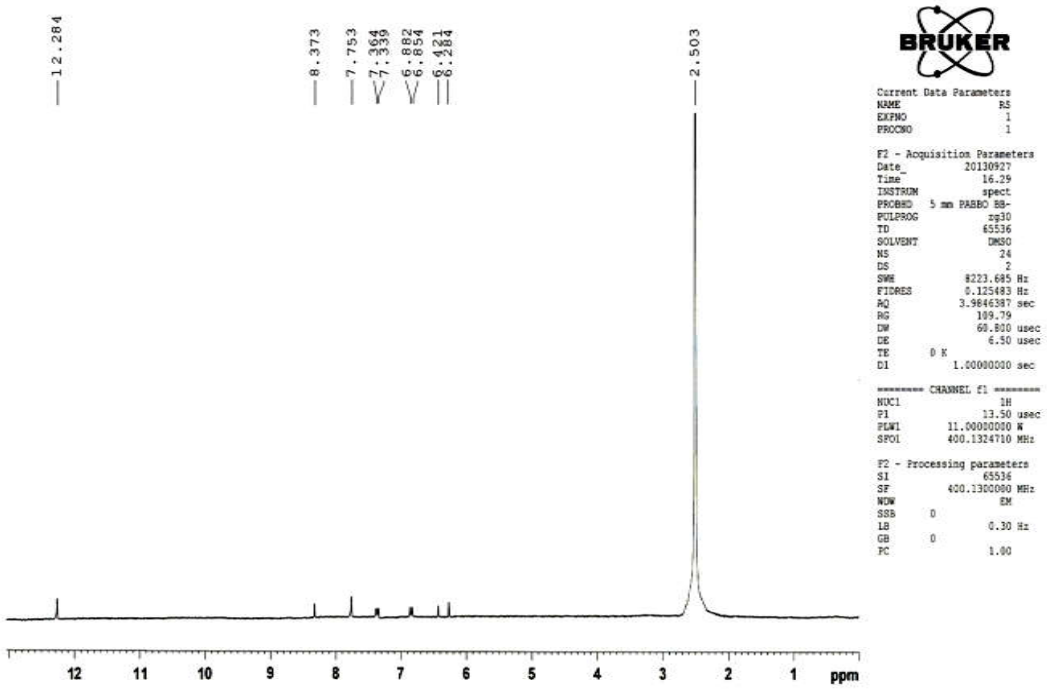


Figure 4 ^{1}H NMR spectrum of Compound

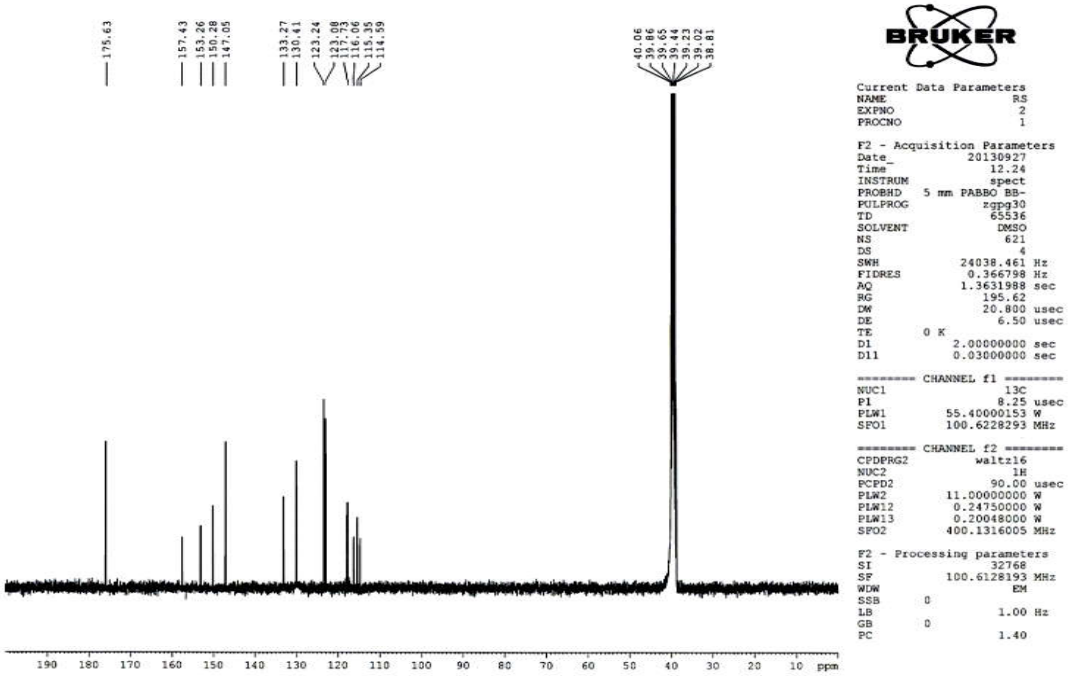


Figure 5 ^{13}CNMR spectrum of Compound

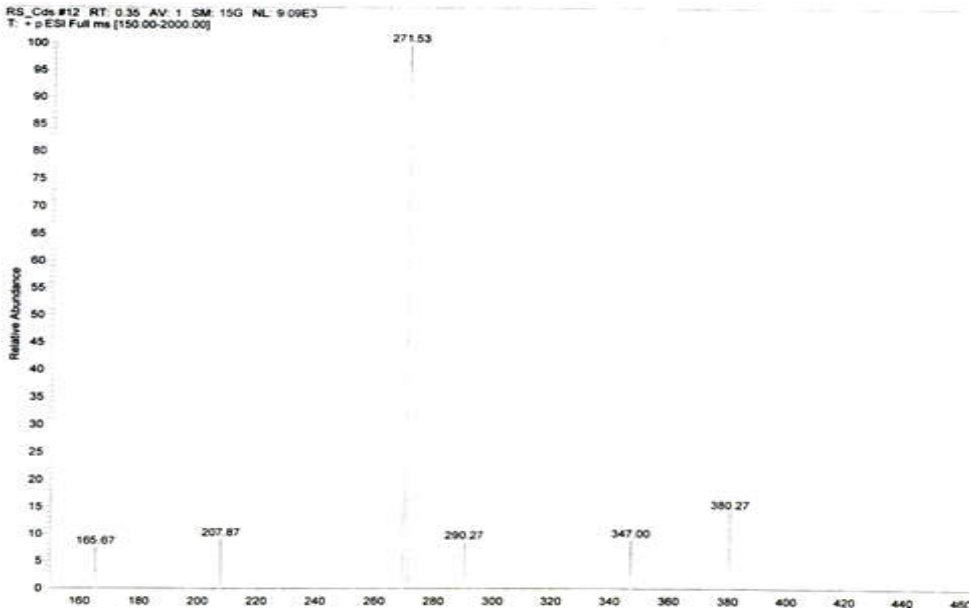


Figure 6 Mass spectrum of Compound

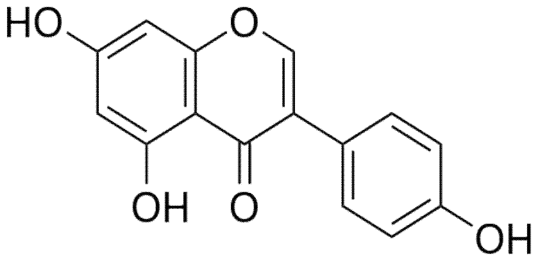


Figure 7 Molecular structure of 5, 7, 4'-trihydroxyisoflavone

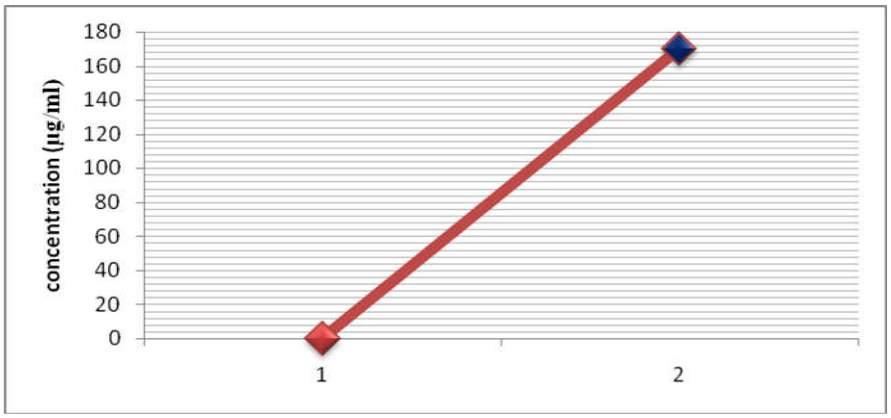


Figure 8 EC_{50} value of Genistein

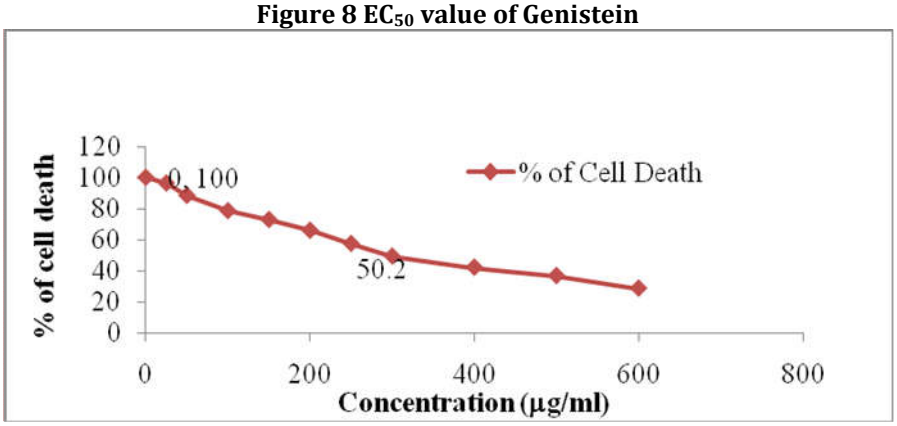
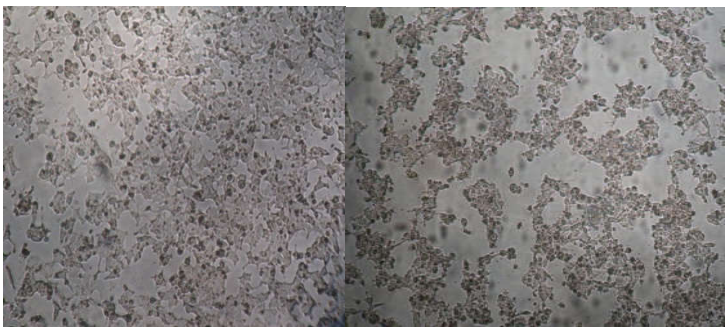


Figure 9 MTT assay on MCF-7 Cell line against the Compound



Without Compound IC50:300µg/ml

Figure 10 MCF-7 Cell line against the compound

CONCLUSIONS

Ipomoea pes-caprae is an effective plant source for traditional drug preparations. One of the purified compound and crude extracts showed good anti-microbial activity [Ethalsha and Malar, 2014]. These findings may be a lead for further ethno-pharmacognostic studies to identify new compounds with therapeutic interest. Many solvent extracts of *Ipomoea pes-caprae* showed biological activity. Furthermore, some solvent extracts were only preliminary studies for their in-vitro activities, so, the advance clinical trial of them deserves to be further investigated. For the first time Genistein has been isolated successfully from the medicinal plant *Ipomoea pes-caprae* under present study. The isolation of the characterised flavanoids would be useful to prepare plant based pharmaceutical preparation to treat various complications linked with human diseases.

REFERENCES

1. Newman DJ, Cragg GM., J. Nat. Prod., 70, 461-77, (2007).
2. Haridas C, Arntzen J, Gutterman A., PNAS., 98, 11557-11562, (2001).
3. Mantle D, Lennard TW, Pickering AT., Adverse Drug Reaciont.Toxicol. Rev., 19, 223-40, (2000).
4. Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT., J. Am. Chem. Soc., 93, 190-198, (1971).
5. Mascarenhas M., Mushroom Research, 3, 77–80, (1994).
6. Devall MS., J. Coastal Research, 8, 442-456, (1992).
7. Kane ME, Bird KT, and Lee TM., J. Coastal Research, 9, 356-362, (1993**).**
8. St. John H., Botanische Jahrbucher Systematik, 89, 563-5831970, (1970).
9. Jirawongse V, Pharadai T, Tantivatana P., J. National Research Council of Thailand, 9, 17-24, (1979).
10. Premanathan M, Nakashima H, Kathiresan K, Rajendran N, Yamamoto N., Indian J. Medical Research, 130, 276-279, (1996).
11. Parekh KK, Patel AM, Modi AJ, Chandrashekhar HR., Pharmacologia, 3, 377-386, (2012).
12. Ethalsha P and Malar Retna A, WJPS., 3, 622-627, (2015).
13. Stahl E., Thin Layer Chromatograpy (A Laboratory Hand Book). 2nd edition, Springer, New York, pp, 856, 883, (1969).
14. Ethalsha P and Malar Retna A, International Journal of Natural Products Research, 4, 58-64 (2014).
15. Mosmann T., J. Immun. Methods, 65, 55–63, (1983).

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