Original Article

Biofuel Production, Phytochemical Analysis And In Silico Analysis For Active Compound Valorization From Jatropha Gossypifolia Seeds

Dowluru SVGK Kaladhar[1,*], Satish Kumar Kenar[2], Sudhakar Meesala[3], Sanju Sahu[4]

[1,2,3,4] Microbiology and Bioinformatics, UTD, Atal Bihari Vajpayee Vishwavidyalaya, Bilaspur (CG), India
Corresponding Author Email, dkaladhar@gmail.com
DOI: 10.47750/pnr.2023.14.02.200

Abstract

Jatropha belongs to family of Euphorbiaceae, is an alternate or commercial crop for biodiesel production. Fresh seeds of Jatropha gossypifolia were collected during summer season (April 2022) and were dried in the sunlight for 24 hours. Around 42ml of the oil was extracted from 100 gm of seeds at 210°C from dried seeds of Jatropha gossypifolia. The seed waste with Saccharomyces cerevisiae was kept for Solid State Fermentation (SSF) for one week. Ethanol with substrate and Saccharomyces cerevisiae were used for extraction of oil after one week. The Jatropha gossypifolia seed oil contains Protein, Carbohydrate, phenol, Terpenoids, steroids, Cholesterol, Quinones, flavonoids and Cardiac glycosides. The seed oil has shown little activity with Candida, Enterococcus, Pseudomonas and Staphylococcus. Antimicrobial activity was observed with Petroleum ether or Ethanol dissolved with microbial oil (50:50).The proteinases like Trypsin and Proteinase K has shown gelatin degradation indicates the proteases making positive effect with gelatin degradation and protein degrading activity. Gossypiline has shown good binding activity showing good antimicrobial activity compared with gossipidien acid, gossipifan, and n - demethylricinine based on in silico analysis.

Keywords: Jatropha gossypifolia, seed oil, Biofuel, in silico

Introduction

Jatropha belongs to family of Euphorbiaceae. The seed oil of Jatropha plant is an alternate or commercial crop for biodiesel production (Banerji et al., 1985; de Oliveira et al., 2009). Jatropha plants are not an edible crop and are used in biofuel production. Jatropha plant is having high potential due to the technical and economic reasons of usage all around the world. Apart from biodiesel production from Jatropha seeds, the various parts of Jatropha plant like leaves, stem, roots, etc., can find useful in different areas like polymeric materials, medicine, fertilizer, fence, etc.

The family Euphorbiaceae is considered as one of the world's largest families of Angiosperms that shows about 7,800 species with approximately 300 types of genera and 5 subfamilies all around the world. The name "Jatropha" has been derived from the Greek words "jatros," meaning as "doctor" and "trophe," meaning as "food," that is connected with good medicinal uses. These species occur favorably in tropical and subtropical regions that are found in Africa, South America, Central America, India, West Indies, and the Caribbean. Jatropha species are using in traditional and modern medicines in Africa, Asia, and Latin America to cure various ailments. Different parts of Jatropha species, such as the leaves, roots, stems, seeds, and latex, are using in medicine (Wu et al., 2019). In India, J. gossypiifolia is being used as a local/ traditional and modern medicine in the treatment of diarrhea dysentery (Bhagat and Kulkarni, 2014), anti-inflammatory, antimicrobial, antineoplastic, antioxidant, anticholinesterase, and antihypertensive activities (Abreu et al., 2003; Panda et al., 2009).

Material and Methods

COLLECTION OF SEEDS

Fresh seeds of J. gossypiifolia was accumulated locally from Bilaspur (CG) region during summer season (Figure 1 and 2).

Figure 1: J. gossypiifolia Plant

.

Figure 2: J. gossypiifolia seeds

Biofuel production

The seeds were air-dried and was used for oil extraction. Oil extraction machine was used for the production process of Biofuel.

Microbial Biofuel production

The substrate obtained after the oil extraction was mixed with the yeast and remained for one week. The substrate + yeast sample was kept for oil extraction along with ethanol. The oil obtained was tested again with flaming.

Phytochemical analysis

The extracted seed oil of J. gossypiifolia was tested for the phytochemical analysis to understand about the chemical components present in the oil (Kaladhar et al., 2014).

X-ray film analysis for protein inhibition activity

Activity for protease inhibitor against proteases (proteinase K and trypsin) was assayed according to the procedure experimented by Kunitz 1947, with small modifications in the procedure (Kunitz, 1947; Dunn, 1989). Approximately 10 µl of protease inhibitor (oil) was mixed with 10 µl of protease (0.5 mg/ml) and was spotted onto a stripe of the X-ray film. 10µl of protease was mixed with 10µl of 0.lM (pH 7.0) phosphate buffer as the control and was spotted on to the X-ray film. The above inhibitor, protein and buffer mixtures were incubated of X-ray film at 37°C for 10 minutes. After 10 minutes, the film was washed under tap water gently without touching other objects for the zone of gelatin hydrolysis. The formation of clear zone as indicator of protease activity which may be due to the hydrolysis of gelatin on X-ray film.

Antimicrobial activity

Microorganisms

Microbes from MTCC (Microbial Type Culture Collection) have been used in the present study. Various bacteria used in the present research work are Staphylococcus aureus (MTCC 740) and Enterococcus faecalis (MTCC 439) belongs to gram positive bacteria and Pseudomonas aeruginosa MTCC 424 belong to gram negative bacteria. Fungi used in the work are Candida albicans MTCC 227.

The bacteria were grown in Muller-Hinton media (HiMedia Pvt. Ltd.,Mumbai., India) at 37°C for 24 hours and fungi in Sabourand Dextrose Media (Himedia Pvt. Ltd.,Mumbai., India) at 25°C for 72 hours, and were maintained on nutrient agar slants at -20°C. Inoculum of test organisms was prepared by growing pure isolate in nutrient broth for overnight. The overnight

broth cultures were sub cultured in fresh nutrient broth and grown for 3 hours to obtain log phase culture. The agar plates were prepared by pour plate method using Muller- Hinton agar (MHA) medium for bacteria and Sabourand Dextrose agar (SDA) Media for fungi. The sterile MHA/SDA medium cooled to 45°C and mixed thoroughly with 1ml of growth culture of concerned test organism ($1x10^8$ cells) and then poured into the sterile petri dishes and allowed to solidify. Wells of 8mm size were made with sterile borer and test extracts were added. The MHA plates were incubated at 37°C for 24 hrs for bacteria. The SDA plates were incubated at 25°C for 72 hrs for fungi. The diameter of zones of inhibition was measured in mm using HiMedia zone reader (Kaladhar et al., 2014).

Docking

Ligands (Figure 3) were designed by ChemSketch v10.03 and receptors (Figure 4) were retrieved from PDB database. iGEMDOCK v2.1 are the free software that has good Protein-Ligand activity. The minimum energy obtained during docking process will be the good ligand. Lower the energy higher will be the stable and effective molecule (Bharat and Kaladhar, 2014; Zengin et al., 2022).

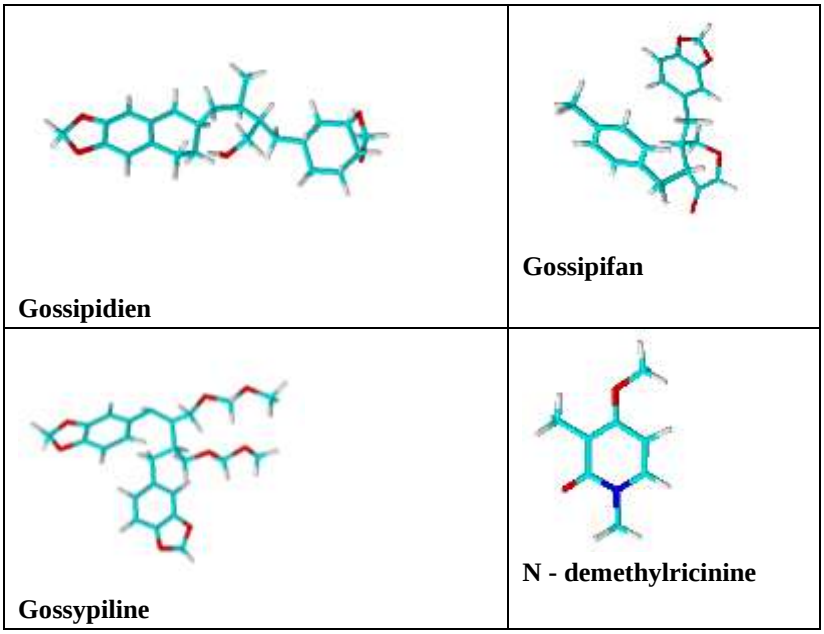
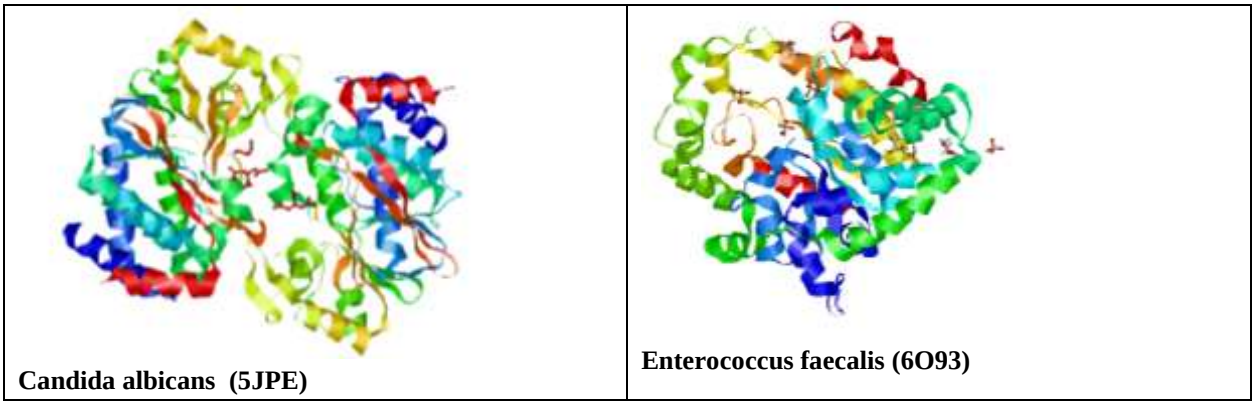
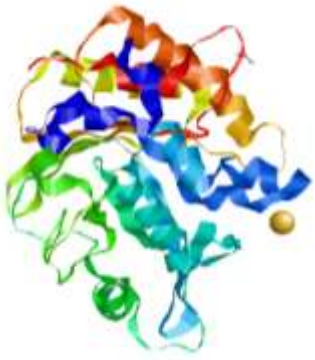


Figure 3: Selected ligands from Jatropha gossypifolia seed oil



Pseudomonas aeruginosa (5WYD)



Staphylococcus pyogenes (3V18)

Figure 4: Selected receptors in present study

RESULTS AND DISCUSSION

Fresh seeds of Jatropha gossypifolia (Figure 5) were collected during summer season (April 2022) and were dried in the sunlight for 24 hours. The dried seeds were kept in the seeds to oil machine and the oil was extracted by heat press method. Around 42ml of the oil was extracted from 100 gm of seeds at 210°C. About 42% seed oil (Figure 6) was obtained from fresh seeds of Jatropha gossypifolia.

Figure 5: Plant and Seeds of Jatropha gossypifolia

% oil extracted= amount of oil in ml/ 100 gm of seed X100= 42/100 X100= 42%

Figure 6: Jatropha gossypifolia Seed oil

Figure 7 shows the extracted seed oil of Jatropha gossypifolia was burned in the sand pot and the oil is burned for about 10 minutes per 10ml of oil (yellow color).

Figure 7: Sand pot used for flame testing

Extracted microbial oil with Jatropha gossypifolia seed/ flower waste material was been used for isolation of oil. The seed waste with Saccharomyces cerevisiae was kept for reaction for one week and was shown in Figure 8.

Figure 8: Jatropha gossypifolia Seed waste substrate mixed with Saccharomyces cerevisiae

Ethanol with substrate and Saccharomyces cerevisiae were used for extraction of oil after one week and was tested in the sand pot for burning. The extract was observed with good orange color with yellow flame (Figure 9).

Figure 9: Microbial oil Vs Seed oil from Jatropha gossypifolia

Phytochemical analysis

Table 1 shows phytochemical analysis of the seed oil of Jatropha gossypifolia.

Protein

To 2 ml of the seed oil of Jatropha gossypifolia, 2 ml of Biuret reagent is to be added. A violet color ring was formed that indicates the presence of protein.

Carbohydrate

To 2 ml of seed oil of Jatropha gossypifolia, add 2 drops of Molisch's reagent and mix the solution. Nearly 2 ml of Conc. H_2SO_4 is to be added drop by drop from the sides of the test tube. A reddish violet color ring appeared at the junction of two layers immediately indicates the presence of carbohydrates.

Amino acid

To 2 ml of the seed oil of Jatropha gossypifolia, 2 ml of Ninhydrin reagent is to be added and keep the solution in hot water bath for 15 minutes. A purple color is not formed that indicates the absence of amino acids in the sample.

Phenols

About 2 ml of seed oil of Jatropha gossypifolia, 3ml of ethanol and a pinch of $FeCl_3$ is to be added. The appearance of greenish yellow color solution indicates the presence of phenols.

Terpenoids

To 2 ml of seed oil of Jatropha gossypifolia, add 2 ml of chloroform and 3 ml of Conc. H_2SO_4. Formation of a monolayer of reddish brown coloration of an interface shows a positive result for the terpenoids.

Steroids

To 2 ml of acetic anhydride, 0.5 ml of seed oil of Jatropha gossypifolia and 2 ml of H_2SO_4 is to be added. The color was changed from green or violet to blue indicates the presence of steroids.

Cholesterol

To 2 ml of chloroform taken in a dry test tube and add 2 ml of the seed oil of Jatropha gossypifolia. About 10 drops of acetic anhydride and 2 to 3 drops of Conc. H_2SO_4 are to be added to the solution. A change from red rose color solution to blue green color solution indicates the presence of cholesterol.

Quinones

To 2 ml of seed oil of Jatropha gossypifolia, 3 ml of Conc. HCl is to be added. Formation of green color solution indicates the presence of quinones.

Flavonoids

To 2 ml of seed oil of Jatropha gossypifolia, 5 ml of dilute ammonia solution, a few drops of Conc. H_2SO_4 are to be added. A yellow colored solution appeared that confirms the presence of flavonoids.

Cardiac Glycosides

To 5 ml of 2 ml of seed oil of Jatropha gossypifolia, add 2 ml of glacial acetic acid contain one drop of ferric chloride solution is to be added. The solution was underlayed with 1ml of Conc. H_2SO_4. A brown colored ring of the edge formed indicates the presence of a deoxysugar is a feature of cardenolides. A violet ring may appear under the brown ring indicates an acetic acid layer and a greenish ring might form just progressively throughout thin layer.

Table 1: Biochemical analysis of Jatropha gossypifolia seed oil

Metabolite	Result
Protein	+
Carbohydrate	++
Amino acid	-
Phenol	+
Terpenoids	++
Steroids	++
Cholesterol	+
Quinones	+
Flavonoids	+
Cardiac Glycosides	+

Note: +++ more positive confirmed ++Average confirmation
+moderate confirmation -negative confirmation

The oil from Jatropha gossypifolia seed oil contains Protein, Carbohydrate, phenol, Terpenoids, steroids, Cholesterol, Quinones, flavonoids and Cardiac glycosides (Table 1).

The antimicrobial activity of the Jatropha gossypifolia seed oil, microbial oil extracts and Gentamycin (Table 2). The seed oil has shown little activity with Candida, Enterococcus, Pseudomonas and Staphylococcus. Less antimicrobial activity was observed with Petroleum ether or Ethanol dissolved with microbial oil (50:50).

Table 2: Antimicrobial activity Jatropha gossypifolia seed oil

Sample	Zone of inhibition (in mm) along with zone size of 8mm			
	Candida	Enterococcus	Pseudomonas	Staphylococcus
Antibiotic (Gentamycin eye drops)	10	16	16	13
Seed oil	10	11	11	12
Petroleum ether mix with microbial oil (50:50)	10	10	10	11
Ethanol dissolved with microbial oil (50:50)	8	8	8	10

X-RAY FILM ANALYSIS FOR PROTEIN INHIBITION ACTIVITY

Trypsin and proteinase K has shown protein degradation with oil (Table 3, Figure 10). The formation of clear zone as indicator of protease activity which may be due to the hydrolysis of gelatin on x-ray film. The oil was not formed zone for gelatin degradation with water. The proteinases like Trypsin and Proteinase K has shown gelatin degradation indicates the proteases making positive effect with gelatin degradation and protein degrading activity. The oil with proteinase has not shown gelatin degradation indicates the oil mix with proteins making negative effect with gelatin degradation. DFO, Burnol, Waxonil, Silvez and Euvolicare has shown negative effect with gelatin degradation.

Table 3: Gelatin degradation Jatropha gossypifolia seed oil

Sample	Gelatin degradation
trypsin	+
Proteinase K	+

Jatropha gossypifolia seed oil + trypsin	-
Jatropha gossypifolia seed oil + Proteinase K	-
Jatropha gossypifolia seed oil	-
DFO	-
Burnol	-
Waxonil	-
Silvez	-
Euvolicare	-
Distilled Water	-

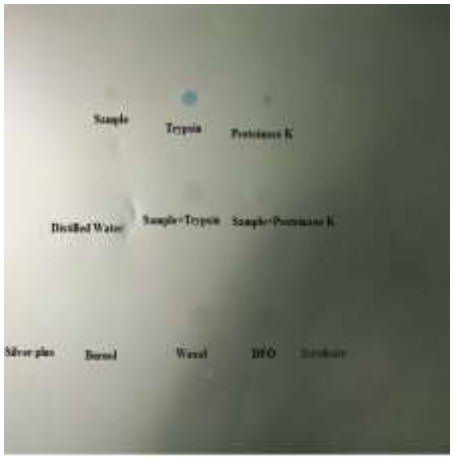


Figure 10: Gelatin degradation activity of Jatropha gossypifolia **seed oil**

Docking

The oil extract collected from plant Jatropha gossypifolia has been searched further compounds based on previous research based on the search result, the selected compounds has been absorbed as gossipidien acid, gossipifan, gossypiline and n - demethylricinine.

Few Jatropha gossypifolia seed compounds has been design as 3D molecule in .mol format using ACD/ChemSketch software v10.02 (Figure 3).

Table 4 shows the QSAR (Quantitative structure-activity relationship) properties shows no partial charges showing molecules as stable. Quantitative structure-activity relationship (QSAR) studies are unquestionably of great importance in modern chemistry and biochemistry. The surface area of a solid object is a measure of the total area that the surface of the object occupies. If temperature and pressure are constant, the number of particles is proportional to the volume. If the hydration energy is greater than the lattice energy, then the enthalpy of solution is negative (heat is released), otherwise it is positive (heat is absorbed). A negative logP value indicates preferential solubility in water and a positive value indicates an affinity for octanol. Refractive index is the ratio of the velocity of light of a specified wavelength in the air to its velocity in the examined substance.As polarizability increases, the dispersion forces also become stronger. Thus, molecules attract one another more strongly and melting and boiling points of covalent substances increase with larger molecular mass. Polarizability also affects dispersion forces through the molecular shape of the affected molecules. Modern mass spectrometers easily distinguish (resolve) ions differing by only a single atomic mass unit (amu), and thus provide completely accurate values for the molecular mass of a compound.

Table 4: QSAR properties of the ligands of Jatropha gossypifolia seed oil

Ligand	**Partial charges (e)**	**Surface Aera (A2)**	**Surface Area (Grid)(A2)**	**Volume (A3)**	**Hydration Energy Kcal/Mol**	**Log P**	**Refractivity (A3)**	**Polarizability (A3)**	**Mass (amu)**
Gossipidien	0.00	485.53	640.99	1131.29	-6.66	2.23	112.93	43.55	402.53

Gossipifan	0.00	451.00	553.52	959.26	-6.41	1.27	102.13	37.06	338.40
Gossypiline	0.00	564.28	659.85	1165.66	-12.59	-0.76	123.84	45.47	444.48
N - demethylricinine	0.00	302.12	310.40	462.00	-9.81	-1.65	39.51	14.55	150.14

The receptor related to disease causing microbes with PDB ids (organism name) has been retrieve and selected as receptor. The ligands and the receptor has been selected in the present experimentation to understand about molecular docking process

The docking result in the present experiment in Table 5. Gossipidien acid, Gossipifan, Gossypiline and N – demethylricinine from Jatropha gossypifolia seed oil are selected as ligands. Gossypiline has shown good binding activity showing good antimicrobial activity compared with gossipidien acid, gossipifan, and n – demethylricinine based on in silico analysis.

Table 5: Docking results for Jatropha gossypifolia seed oil compounds

COMPOUND NAME	Candida	Enterococcus	Pseudomonas	Staphylococcus
Gossipidien	-108.7	-104.8	-104.3	-98.1
Gossipifan	-118.4	-89.8	-96.7	-91.9
Gossypiline	**-124**	**-127.1**	**-106.4**	**-130.1**
N - demethylricinine	-77	-59.3	-67.2	-72.3

The docking of microbial proteins with the gossypiline with Staphylococcus has been presented in figure 11.

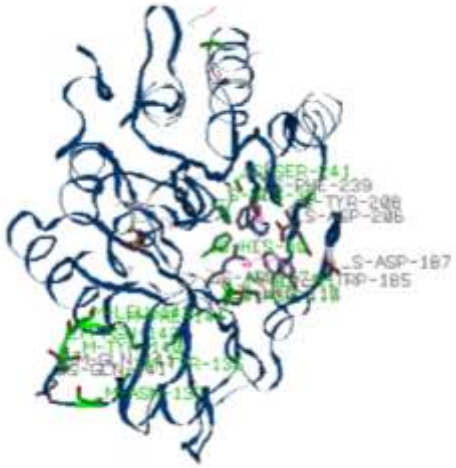


Figure 11: Docking visualization of Gossypiline with Staphylococcus

Several vegetable oil feedstocks including palm oil, coconut oil, peanut, Orbignya sp., canola, turnip, J. curcas, A. aculeata, and almonds, are been considered for biodiesel production in countries like Brazil. According to the fungal growth reports, the stored biodiesels and oils were potentially susceptible to the microbial degradation. Longer test times at pilot scale in susceptibility of these biodiesel feedstock candidates can understand about the shelf life during storage (Makkar and Becker, 2010).

In the present work, J gossifolia seed oil was extracted using heat and press method and had got an yield of 42%. As per Pandey et al., 2012 Jatropha curcas has seed oil content of 33.3% followed by kernel based oil. For the studied species, the mean oil content of J. curcas was 35.0%, 18.3% for J. mollissima and 22.1 % for J. gossypiifolia (Barros et al., 2015)

Jatropha gossypiifolia L. [Euphorbiaceae], widely known as “bellyache bush”, is a medicinal plant native to south America, Mexico, India and many west African countries like Nigeria (Aboaba et al., 2015). Pharmacological studies of Jatropha gossypiifolia L have demonstrated significant action of different extracts and isolated compounds as anticholinesterase, haemostatic, antiinflammatory, antimicrobial, antidiarrheal, antihypertensive, and antiproliferative agents, are supporting to

some of its folkloric uses (Fatokun et la., 2006). The major secondary metabolites isolated from various extracts f Jatropha gossypiifolia L are terpenes-essential oils, alkaloids, coumarin, and lignans (Fatokun et al., 2016).

Conclusion

The dried seeds of Jatropha gossypiifolia L were kept in the seeds to oil machine and the oil was extracted by heat press method. Around 42ml of the oil was extracted from 100 gm of seeds. About 42% seed oil was obtained from fresh seeds of Jatropha gossypiifolia L. the extracted oil was burned in the sand pot and the oil is burned for about 10 minutes per 10ml of oil (yellow color). Ethanol with substrate and Saccharomyces cerevisiae were used for extraction of Jatropha gossypiifolia L oil after one week and was tested in the sand pot for burning. The extract was observed with good orange color with yellow flame. The oil from Jatropha gossypifolia seed oil contains Protein, Carbohydrate, phenol, Terpenoids, steroids, Cholesterol, Quinones, flavonoids and Cardiac glycosides. The seed oil has shown little activity with Candida, Enterococcus, Pseudomonas and Staphylococcus. Gossypiline has shown good binding activity showing good antimicrobial activity compared with gossipidien acid, gossipifan, and n - demethylricinine based on in silico analysis.

Acknowledgement

The authors thank the management of Atal Bihari Vajpayee Vishwavidyalaya, Bilaspur (CG) for providing opportunity to conduct research.

Conflict of interest

There is no conflict of interest

References

1. Aboaba SA, Adebayo MA, Ogunwande IA, Olayiwola TO. Volatile constituents of Jatropha gossypifolia L. grown in Nigeria. American Journal of Essential oils and Natural products. 2015;2(4): 08-11.

2. Abreu IC, Marinho AS, Paes AM, Freire SM, Olea RS, Borges MO, Borges AC. Hypotensive and vasorelaxant effects of ethanolic extract from Jatropha gossypiifolia L. in rats. Fitoterapia. 2003; 74(7-8): 650-657.

3. Banerji R, Chowdhury AR, Misra G, Sudarsanan G, Verma SC, Srivastava GS. Jatropha seed oils for energy. Biomass. 1985; 8(4): 277-282.

4. Barros TFS, Arriel NHC, Queiroz MF, Fernandes PD, Mendonça S, Ribeiro JAA, Medeiros EP. Fatty acid profiles of species of Jatropha curcas L., Jatropha mollissima (Pohl) Baill. and Jatropha gossypiifolia L. Industrial Crops and Products. 2015; 73: 106-108.

5. Bhagat RB, Kulkarni DK. Evaluation of Phytochemical, antibacterial and antidiarrhoel activity of Jatropha Gossypifolia L. Root methanol extract in swiss albino mice. World Journal of Pharmaceutical Research. 2014; 3(4): 566-581.

6. Bugata BK, DSVGK K. QSAR and docking studies of synthesized diarylsulfonylurea chalcone hybrids as antiinflammatory agents. Int J Pharm Sci Rev Res. 2014; 24: 144-9.

7. de Oliveira JS, Leite PM, de Souza LB, Mello VM, Silva EC, Rubim JC, Meneghetti SMP, Suarez PA. Characteristics and composition of Jatropha gossypiifolia and Jatropha curcas L. oils and application for biodiesel production. Biomass and bioenergy. 2009; 33(3): 449-453.

8. Dunn BM. Determination of protease mechanism. Proteolytic enzymes: a practical approach. 1989; 1: 57-81.

9. Fatokun OT, Liberty O, Esievo KB, Okhale SE, Kunle OF. Phytochemistry, Ethnomedicine and Pharmacology of Jatropha gossypiifolia L: A Review. Phytochemistry., 2016;5(3): 1-21.

10. Gurib-Fakim A. Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular aspects of Medicine. 2006:27(1): 1-93.

11. Kaladhar DSVGK, Duddukuri GR, Yarla NS. Phytochemical analysis, antioxidant and antimicrobial activities from raw fruit peel crude extracts of Annona squamosa Linn. World Journal of Pharmacy and Pharmaceutical Sciences, 2014; 4(1): 1373-1380.

12. Kunitz M. Crystalline soybean trypsin inhibitor: II. General properties. The Journal of General Physiology. 1947; 30(4): 291-310.

13. Makkar HP, Becker K. Are Jatropha curcasphorbol esters degraded by rumen microbes?. Journal of the Science of Food and Agriculture. 2010; 90(9): 1562-1565.

14. Panda BB, Gaur K, Kori ML, Tyagi LK, Nema RK, Sharma CS, Jain AK. Anti-inflammatory and analgesic activity of Jatropha gossypifolia in experimental animal models. Global journal of pharmacology. 2009; 3(1): 1-5.

15. Wu Q, Patocka J, Nepovimova E, Kuca K. Jatropha gossypiifolia L. and its biologically active metabolites: A mini review. Journal of ethnopharmacology. 2019; 234: 197-203.

16. Zengin G, Mahomoodally MF, Yıldıztugay E, Jugreet S, Khan SU, Dall'Acqua S, Mollica A, Bouyahya A, Montesano D. Chemical Composition, Biological Activities and In Silico Analysis of Essential Oils of Three Endemic Prangos Species from Turkey. Molecules. 2022; 27(5):1676.