IJIRR

International Journal of Information Research and Review
Vol. 1, Issue, 10, pp. 124-127, October, 2014

Full Length Research Article

PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF VARIOUS EXTRACTS OF CASTOR FRUIT-SEEDS (*RICINUS COMMUNIS* L)

[1,*]Asish Bhaumik, [2]Swapna, M., [3]Sucharitha, M., [4]Sravya, S., [5]Lavanya, M. and [6]Siraj Ahmed Md

[1, 2, 3]Department of Pharmaceutical Chemistry, Teja College of Pharmacy, Kodad, Nalgonda-508206, Telangana State, India
[4, 5]Department of Pharmaceutical Biotechnology, Teja College of Pharmacy, Kodad, Nalgonda-508206, Telangana State, India
[6]Department of Pharmaceutics, Anurag Pharmacy College, Kodad, Nalgonda-508206, Telangana State, India

ARTICLE INFO

Article History:
Received 25th July, 2014
Received in revised form
05th August, 2014
Accepted 04th September, 2014
Published online 30th October, 2014

Keywords:
Ricinus Cmmunis L,
Perennial Bushy,
Anti Bacterial Activity,
Anti Fungal Activity,
Mic etc.

ABSTRACT

The castor oil plant is a fast-growing, suckering perennial shrub or occasionally a soft wooded small tree up to 6 meter or more, but it is not hardy in nature. Two varieties of this plant are known a perennial bushy plant with large fruits and large red seeds which yields about 40 P.C of oil. A much smaller annual shrub with small grey (white) seeds having brown spots and yielding 37% of oil. It is one of the most important fruits worldwide because the castor oil obtained from the seed of the plant is still widely used traditionally and herbally as a medicine. Recently the crude whole fruits with seeds had shown that potential biological activities against various infectious diseases. The objectives of the present work was to search antimicrobial activities. Based on this, a new series of constituents had been planned to extract by methanol (E1), ethanol (E2), acetone (E3), chloroform (E4) and ether (E5) from castor fruit-seeds. The *in-vitro* antimicrobial activity of various extracts of fruit-seeds of R. communis were carried out by using agar diffusion method using bacterial cultures *Staphylococcus aureus (ATCC 9144), Bacillus subtilis (ATCC 6633), Pseudomonas aeruginosa (ATCC 27853,) Escherichia coli (ATCC 25922)* and fungal cultures *Aspergillus niger (ATCC 9029), Aspergillus flavus (ATCC 204304), Candida albicans (ATCC 10231)*. By observing it was found that most of the extracts executed moderate to good antimicrobial activity against the tested micro-organisms. The extracts were active against all the tested microorganism for anti bacterial activity with range of MIC values for *S.aureus* (MIC: 15-39 µg /ml), *E.coli* (MIC: 16-38 µg /ml), *P.aeruginosa* (MIC:15-39 µg /ml) and *B.subtilis* (14-39 µg /ml). The extracts were active against all the tested microorganism for anti fungal activity with the range of MIC values for *A.niger* (MIC :17-39 µg/ml),*A.flavus* (18-37 µg/ml) and *C.albicans* (16-35 µg/ml).

INTRODUCTION

The castor oil plant is a fast-growing, suckering perennial shrub or occasionally a soft wooded small tree up to 6 meter or more, but it is not hardy in nature. This plants was cultivated for leaf and flower colors and for oil production. Leaves are green or reddish in colour and about 30-60 cm in diameter. The leaves contain 5-12 deep lobes with coarsely toothed segments which are alternate and palmate. The stems are varying in pigmentation. The flowers are monoecious and about 30-60 cm. long (The Wealth of India, 1972).The fruit is a three-celled thorny capsule. The capsule of fruit covered with soft spins like processes and dehiscing in to three 2-valved cocci. The seeds are considerable differences in size and colour. They are oval, somewhat compressed, 8-18 mm long and 4-12 mm broad. The testa is very smooth, thin and brittle. Castor seeds have a warty appendage called the caruncle, which present usually at one end from which runs the raphe to terminate in a slightly raised chalaza at the opposite end of the seed (Trease, 2002).

The castor oil obtained from the seed of the plant is still widely used traditionally and herbally as a medicine. The seed of the plant is used as fertilizer after the oil was extracted cooked to destroy the toxin and incorporated into animal feeds. The

Corresponding author: Asish Bhaumik
Department of Pharmaceutical Chemistry, Teja College of Pharmacy, Kodad, Nalgonda-508206, Telangana State, India

principal use of castor oil is as a purgative and laxative. It is also used as a lubricant, lamp fuel, a component of cosmetics, and in the manufacture of soaps, printer's ink, plastics, fibers, hydraulic fluid, brake fluid, varnishes, paints, embalming fluid, textile dyes, leather finishes, adhesives, waxes, and fungicides. In India, the leaves are used as food for eri silk worms and the stalks are used for fuel purpose. This species has been planted for its dune stabilization properties (Encyclopedia Britanica, 2000; CSIR, 1972 and Kadambi and Dabral, 1955). The Preliminary Phytochemical study of *R. communis* presence of steroids, saponins, alkaloids, flavonoids, and glycosides.

The dried leaves of R. communis showed the presence of alkaloids, ricinine (0.55%) and N demethylricinine flavones glycosides kaempferol-3-O and kaempferol-3-O-β-D-glucopyranoside, quercetin, xylopyranoside quercetin ,kaempferolO-β-rutinoside (Kang *et al*., 1985) and quercetin-3-O-β- monoterpenoids (1, 8-cineole, camphor and α sesquiterpenoid (β-caryophyllene), gallic acid, quercetin, gentisic acid, rutin, epicatechin and ellagic acid are the major phenolic compounds isolated from leaves. Indole-3-acetic acid has been extracted from the roots (Darmanin *et al*., 2009; Singh and Ambika Chauhan, 2009)**.** The seeds contain 45% of which consist glycosides of ricinoleic, isoricinoleic, stearic and dihydroxystearic acids and also lipases and a ricinine (Khogali *et al*., 1992)**.** The GLC study of castor oil showed the presence form of palmitic (1.2%), stearic (0.7%), arachidic (0.3 hexadecenoic (0.2%), oleic (3.2%), linoleic (3.4 ricinoleic (89.4%) and dihydroxy stearic acids (Kang *et al*., 1985)**.** The stem also contains ricinine.The ergost-5-en-3-ol, stigmasterol, Y- sitosterol, fucosterol and one probucol isolated from ethr extract of seeds. The GC-MS analyses of R.communis essential oil using capillary columns are identified compounds like α-thujone (31.71%), and 1,8 cineole(30.98%) ,α-pinene (16.88%),camphor (12.92%) camphene (7.48%) (Kadri *et al.,* 2011) and lupeol and 30–norlupan-3β-ol-20-one are obtained from coat of castor bean (Malcolm J. Thompson and William S. Bowers, 1968).

The literature survey revealed that the various extracts of *R. communis* were possessed a wide range of pharmacological activities like, Antioxidant, Hpatoprotective, Antihistaminic, Antiasthamatic, Anti inflammatory, Antifertility, Antimicrobial, Antidiabetic, Antiulcer, lipolytic, in vitro immunomodulatory, wound healing, Insecticidal and Larvicidal etc. The number of infections which are caused by multi drug resistant gram positive and gram negative pathogens and viruses are life threatening for human being.Infections caused by these organisms pose a serious challenge to the scientific community and need for a effective therapy has lead for novel antimicrobial agents

MATERIALS AND METHODS

Drugs and chemicals

Standard drug Cprofloxacin (Antibacterial) and Ketoconazole (Antifungal) were purchased from Local Retail Pharmacy Shop and solvents and other chemicals were used from Institutional Store and were of AR grade. Bacterial cultures *Staphylococcus aureus* **(***ATCC 9144), Bacilus subtilis (ATCC 6633), Pseudomonas aeruginosa (ATCC 27853,) Escherichia coli (ATCC 25922)** and fungal cultures *Aspergillus niger (ATCC 9029), Aspergillus flavus (ATCC 204304), Candida albicans (ATCC 10231)* were provided by the Biotechnology Lab of the CLBMCP, Chennai and maintained on Nutrient agar slant and fungal strains were maintained on Sabouraud dextrose broth at 4^0C.

Extraction (Raj K. Bansal, ?)

Weigh 50 g of castor fruit-seeds (unripe can be mashed to prepare a paste) into a 500 ml round-bottomed flask. Add 200 ml of methanol. Heat the mixture under reflux for 5 min on stem-bath with frequent shaking. Filter the mixture under suction and transfer the filtrate to a separatory funnel. Wash this mixture containing bioactive compounds with three portions of 250 ml each with sodium chloride solution. Dry the organic layer over anhydrous magnesium sulfate. Filter and evaporate most of the solvent in vacuum without heating. The paste of castor fruit-seeds extracted with Ethanol (E2), Acetone (E3), Chloroform (E4), and Ether (E5) from *R.communis* and followed same procedure.

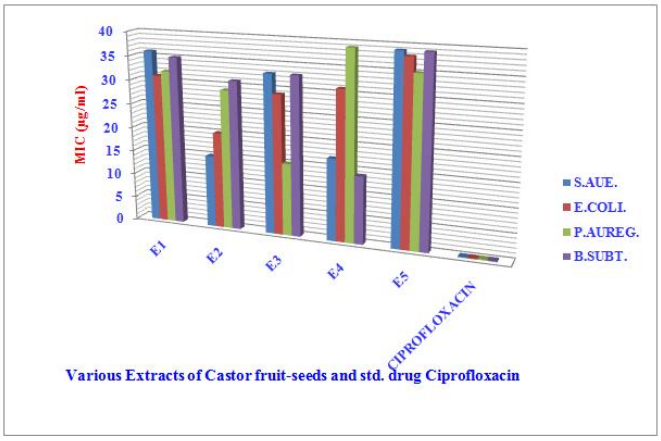

Fig. 1. For graphical representation of MIC (µg/ml) of various extracts of castor fruit-seeds against different Bacteria

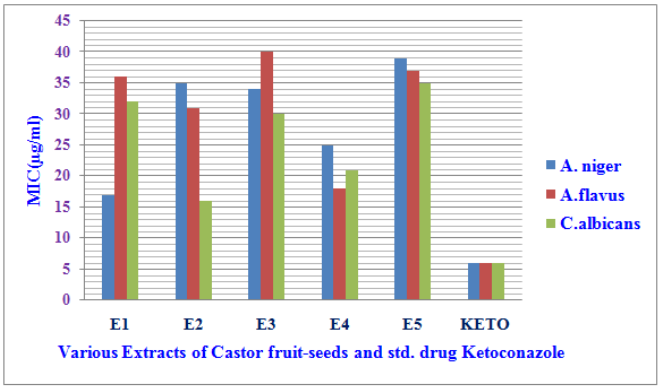

Fig. 2. For graphical representation of MIC (µg/ml) of various extracts of castor fruit-seeds Against different Fungi.

Preliminary Phytochemical Screening (Dandiy and Sharma, ?; Jaswant Kaur, 2010)

Preliminary Phytochemical Screening of various extracts of fruit-seeds of *R.communis* have shown the presence of following bioactive compounds such as reducing sugars, disaccharides, polysaccharides (E1, E2, E3) amino acids (E1, E2, E3), phytosterols (E4, E5), polyphenols (E4, E5) and

Table 1. For zone of Inhibition various Extracts of castor fruit-seeds (*Ricinus communis)* (mm) for Antibacterial activity

Extracts	S. auereus			E.Coli			P.aeruginosa			B.subtilis		
	Concentration (µg/disc)											
	50	100	150	50	100	150	50	100	150	50	100	150
E1	15	19	22	16	20	24	14	18	25	11	16	19
E2	19	25	34	18	24	33	17	22	30	16	20	29
E3	16	22	30	16	21	29	16	19	27	15	17	26
E4	18	24	33	17	23	32	16	21	29	16	18	27
E5	10	16	19	12	15	19	13	19	23	14	18	24
Ciprofloxacin	25	30	39	26	32	40	24	32	38	26	31	38

Table 2. For Minimum Inhibitory Concentration of various Extracts of Castor fruit-seeds (*Ricinus communis)* (Bacteria)

Name of the Extracts	Minimum Inhibitory Concentration (µg/ml)			
	S.auereus	E.coli	P.aerug	B.subt
E1	36	31	32	35
E2	15	20	29	31
E3	33	32	15	33
E4	17	16	39	14
E5	39	38	35	39
Ciprofloxacin	0.2	0.2	0.2	0.2

Table 3. For zone of Inhibition various Extracts of castor fruit-seeds (*Ricinus communis)* (mm) for Anti fungal activity

Name of the Extracts	A. niger			A.flavus`			C.albicans		
	CONCENTRATION (µg/disc)								
	50	100	150	50	100	150	50	100	150
E1	19	20	23	20	20	22	18	20	22
E2	18	19	21	19	19	21	17	19	21
E3	17	16	19	16	18	19	16	19	20
E4	20	22	24	21	22	23	19	21	23
E5	17	14	15	16	16	17	15	16	19
Ketoconazole (100µg/ml	38	38	38	35	35	35	36	36	36

Table 4. For Minimum Inhibitory Concentration of various Extracts of Castor fruit-seeds (*Ricinus communis)* (Fungi)

Name of the Extracts	Minimum Inhibitory Concentration (µg/ml)		
	A.niger	A.flavus	C.albicans
E1	17	36	32
E2	35	31	16
E3	34	40	30
E4	25	18	21
E5	39	37	35
Ketoconazole	6.1	6.1	6.1

Evaluation of Antimicrobial Activity by paper disc diffusion method (Paniker, 2006; Vibhor K Jain *et al.*, 2010; Gaud and Gupta, 2004)

The sterilized (autoclaved at 120^{o}C for 30 min) medium was inoculated (1mL/100mL of medium) with the suspension [10^5 cfu m/l (colony forming unit per milliliter)] of the microorganism (matched to McFarland barium sulphate standard) and poured in Petridish to give a depth of 3-4mm. The paper impregnated with the test compounds (50, 100,150 µg/ml in dimethyl formamide) was placed on the solidified medium. The plates were pre-incubated for 1hr at RT and incubated at 37 ^{o}C for 24 hr for anti-bacterial and antifungal activities respectively. Cprofloxacin (100 µg/disc) and Ketoconazoloe (100 µg/disc) was used as a standard.

Determination of MIC by agar streak dilution method (Hawkey and Lewis, 1994)

MIC of the various extracts of **castor fruit-seeds** were determined by agar streak dilution method. A stock solution of the extracts (100µg/ml) in Dimethylformamide were prepared and graded quantities of the test extracts were incorporated in specified quantities of molten nutrient agar medium. A specified quantity of the medium containing the compounds was poured into a Petri dish to give a depth of 3-4mm and allowed to solidify. Suspension of the micro-organism were prepared to contain approximately10^5 cfu m/l and applied to plates with serially diluted extracts in Dimethylformamide to be tested and incubated at 37^{o}C for 24hr. for bacteria and fungi. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria on the plate.

RESULTS AND DISCUSSION

The phytochemical screening of various extracts of castor fruit-seeds were carried out by using standard procedure and the presence bioactive compounds in various extracts of castor fruit-seeds were confirmed by their specific qualitative chemical confirmatory tests. The zone of inhibition of various extracts of castor fruit-seeds (*Ricinus communis)* were compared with the standard drug ciprofloxacin for the anti bacterial activity and Ketoconazole for the Anti fungal activity and Minimum Inhibitory Concentration (MIC) of various extracts of castor fruit-seeds for bacteria and fungi were shown in Table-1, 2, 3, and 4. The various extracts of castor fruit-seeds *(R.communis)* were possessed both anti bacterial and anti fungal activity. These activities were mainly due to the presence of bioactive compounds in the endocarp of the fruit like amino acids, such as aromatic and α-amio acids, phytosterol, polyphenols, flavanoids, alkaloids etc. The various extracts of castor fruit-seeds *(R.communis)* were (50, 100 and 150µg/ml) screened for antimicrobial activity by paper disc diffusion method. The experimental data had shown that most of the extracts executed moderate to good antimicrobial activity against the tested micro-organisms. The MIC of the various extracts were screened by agar streak dilution method. The experimental data had shown that most of the extracts were active against all the tested microorganism for anti bacterial activity with range of MIC values for *S.aureus* (MIC: 15-39 µg /ml), *E.coli* (MIC: 16-38 µg /ml) ,*P.aeruginosa* (MIC:15-39 µg /ml) and *B.subtilis* (14-39 µg /ml) and the extracts were active against all the tested microorganism for anti fungal activity with the range of MIC values for *A.niger* (MIC :17-39 µg/ml), *A.flavus* (18-37 µg/ml) and *C.albicans* (16-35 µg/ml).

Conclusion

By observing it was found that most of the extracts (E1, E2, E3, E4 and E5) exhibit moderate to good antibacterial activity with an range of MIC between 15-39 µg/ml and antifungal activity with an range of MIC between 16-39 µg/ml. It was found that the extracts E1, E2 and E3 and E4 were exhibited good antibacterial activity and the extracts E1, E2 and E4 were exhibited good antifungal activity.

REFERENCES

CSIR. 1972. The wealth of India. Raw materials. Vol. 9.Publications and Information Directorate, Council for *Scientific and Industrial Research*, New Delhi. 472.

Dandiya, P.C., Sharma, P. K. Bio-chemistry and clinical pathology, second edition, PP- 17-18, 24, 47-48.

Darmanin S, Wismaver PS, Camillerri Podesta MT, Micallef MJ, Buhagiar JA. An extract from Ricinus communis L. leaves possesses cytotoxic properties and induces apoptosis in SKMEL- 28 human melanoma cells. Nat *Prod Res.,* 2009; 23(6): 561-571.

Encyclopedia Britanica. 2000. Castor oil. http://www.Britanica.com/bcom/eb/article/4/0,57.16, 2105+1+20724,00htm? query=castoroil%20 oil.

Gaud, R. S., Gupta, G. D. "Practical microbiology". Nirali prakashan. Mumbai 3rd ed. 2004: 41.

Hawkey BY, Lewis DA, Medical microbiology- a practical approach. United kingdom: Oxford university press; 1994. 181-94.

Jaswant Kaur, PV Chemistry of Natural Products, 2010 edition, PP-113-114, 116, 344-346, 381.

Kadambi, K. and S.N. Dabral. 1955. The silviculture of *Ricinus communis* Linn. *Indian Forester,* 81(1): 53-58.

Kadri Adel.; Gharsallah Neji.; Damak Mohamed.; Gdoura Radhouane.; Chemical composition and *in vitro* antioxidant properties of essential oil of *Ricinus communis* L. *Journal of Medicinal Plants Research,* Vol. 5(8), pp. 1466-1470 18 April, 2011.

Kang SS, Cordell A, Soejarto DD, Fong HHS. 1985. Alkaloids and flavonoids from *Ricinus communis*. *J. Nat. Prod*., 48 (1), 155–156.

Kang, S.S., Cordell, A., Soejarto, D.D., Fong, H.H.S., 1985. Alkaloids and flavonoids from *Ricinus communis*. *J. Nat. Prod*., 48 (1), 155–156.

Khogali A, Barakat S, Abou-Zeid H. 1992. Isolation and identification of the phenolics from *Ricinus communis* L. *Delta J. Sci.,* 16, 198–211.

Malcolm J. Thompson, William S. Bowers.; Lupeol and 30-norlupan-3β-ol-20-one from the coating of the castor bean (*Ricinus communi* L.); *phytochemistry* 1968, 7, 845-847.

Paniker, K. J., Ananthanarayan and Paniker's. "Text-book of microbiology''. 7th ed 2006: 628-630.

Raj.K. Bansal, Laboratory manual of organic chemistry , 5th revised edition , PP- 238-239.

Singh PP, Ambika Chauhan SMS. Activity guided isolation of antioxidants from the leaves of Ricinus communis L. *Food Chem* 2009; 114(3): 1069-1072.

The Wealth of India. A Dictionary of Indian Raw Material and Industrial Products, Vol-IX, 1972, 26-47.

Trease, G.F and Evans, W.C. 2002. Pharmacognosy, 15th Ed. Saunders.

Vibhor K Jain, Sudeep Mandal, Dibyajyoti Saha,, Bindu Jain."Synthesis, characterization and evaluation of antibacterial and antifungal activity of triazole derivatives of gallic acid" Ijabpt 2010; 1 (3): 1300-1311.

******