

DEVELOPMENT OF FORMULATION CONTAINING EXTRACTS OF *ARISTOLOCHIA BRACTEATA* AND *CASSIA TORA* – EVALUATION OF ANTIMICROBIAL ACTIVITY

JAYASUTHA J^{1*}, MONIC JOSEPHINE NITHILA S², VEERABATHINI RAJINIKANTH²

¹SRM College of Pharmacy, SRM University, Kattankulathur, Kancheepuram, Tamilnadu, India, ²Brown's College of Pharmacy, Khammam, Andhra Pradesh, India Email: jayasutha15@gmail.com

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ABSTRACT

The aqueous and ethanolic extract of *Aristolochia bracteata* and *Cassia tora* was screened for antimicrobial activity having the concentration of 2 mg, 4 mg and 6 mg by disc diffusion method, using nutrient agar medium against Gram positive bacteria such as *Staphylococcus aureus*, *Staphylococcus albus*, *Bacillus subtilis*, *Streptococcus faecalis* and Gram negative bacteria such as *Escherichia coli*, *Klebsiella aerogenes*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. Then the extract also tested against fungi *Candida albicans* and *Aspergillus niger*. *Ciprofloxacin* and *Clotrimazole* are used as a reference standard for bacteria and fungi respectively. Among the two extracts of *Aristolochia bracteata* and *Cassia tora*, ethanol extract was found to have the significant activity against bacteria. Nil effect at 2 & 4mg levels and very minimum effect at 6mg level produced by the aqueous extracts of both the plants. Antifungal activity assessment also indicates that the tested fungal strains are more susceptible to aqueous extract of both the plants.

Keywords: *Aristolochia bracteata*, *Cassia tora*, Antibacterial activity, Antifungal activity

INTRODUCTION

Aristolochia bracteata is used in traditional medicine as a gastric stimulant and in the treatment of cancer, lung inflammation, dysentery and snake bites¹. This plant belongs to the family Aristolochiaceae. It has insecticidal properties. Its roots and leaves are bitter and antihelminthic, and are medicinally important. Almost every part of the plant has medicinal usage. Identifying bioactive compounds and establishing their health effects are active areas of scientific enquiry². *Cassia tora* (Leguminosae) is a wild crop and grows in most parts of India as a weed. According to ayurveda, the leaves and seeds are acrid laxative, antiperiodic, anthelmintic, ophthalmic, liver tonic, cardiogenic and expectorant³. The leaves and seeds are useful in leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, cardiac disorders⁴. The present study aims to determine the antimicrobial activity of *Aristolochia bracteata* and *Cassia tora*.

MATERIALS AND METHODS

Collection of the plant materials

The plants *Aristolochia bracteata* and *Cassia tora* is widely found throughout India. For our work the plants were collected in and around Thanjavur, Tamilnadu, India. The leaves were collected and they are dried in room temperature. Then they are crushed in to small pieces and these dried small pieces are finely powdered.

Extraction

The extraction is done by maceration process and the powder material was extracted with water and ethanol. About one hundred grams of the powdered leaf material was mixed with four hundred ml (1:4) of the above solvents and subjected to extraction by cold maceration method. The set up were kept at room temperature for seven days for complete extraction during the period of maceration, the content was shaken occasionally to mix and enhance extraction. After seven days the extract were filtered through muslin cloth. The extract was collected in suitable container and it was subjected to concentration by evaporation.

Preliminary qualitative phytochemical examination of aqueous and ethanolic extracts of *Aristolochia bracteata* and *Cassia tora*

Both the extracts were evaluated for preliminary phytochemical screening for identification of its active constituents like alkaloids, glycosides, phytosterol, fixed oils and fats, saponins, tannins and phenolic compounds. Proteins and free amino acids, gums, mucilages, flavonoids, lignin.

Antimicrobial Screening

The aqueous and ethanolic extracts of *Aristolochia bracteata* and *Cassia tora* was screened for antimicrobial activity by disc diffusion method, using nutrient agar medium against Gram positive bacteria such as *Staphylococcus aureus*, *Staphylococcus albus*, *Bacillus subtilis*, *Streptococcus faecalis* and Gram negative bacteria such as *Escherichia coli*, *Klebsiella aerogenes*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. Then the extract also tested against fungi *Candida albicans* and *Aspergillus niger*. *Ciprofloxacin* and *Clotrimazole* are used as a reference standard for bacteria and fungi respectively. The plates were divided in to 5 sections according to the number of standards and sample solution to be used with the help of the glass marker. Sterile cotton swab was dipped in to the inoculums; excess fluid was removed by rotating the swab with firm pressure. The standard disc and the sterile discs loaded with 2, 4 & 6 mg of the extracts were placed into one section of the plate. Then the plates were incubated at 37°C for 18 - 24 hours till perfect growth was observed. The inhibitory zone was measured with the help of an antibiotic zone reader. The corresponding solvents used for extraction acts as control.

RESULTS AND DISCUSSIONS

The plant materials of *Aristolochia bracteata* and *Cassia tora* were selected for the study to evaluate the formulation containing the extracts of these plants for their antimicrobial activity. Aqueous and ethanolic extracts of these plants were made by cold maceration method and the obtained extracts concentrated by evaporation and preliminary phytochemical screening were made for the obtained extracts by standard procedure.

Table 1 reveals the presence of various phytoconstituents. Both aqueous and ethanolic extracts of *Aristolochia bracteata* showed the presence of alkaloids, saponins, phytosterol, fixed oils & fats, proteins and amino acids. Presence of gums and mucilage observed in aqueous and flavonoids in ethanol extract respectively according to their solubility.

Table - 2, reveals the phytoconstituents of *Cassia tora* in aqueous and ethanol extract. Both the extracts showed the presence of alkaloids, carbohydrates, glycosides, phytosterols, fixed oils & fats and flavonoids. The presence of gums & mucilage observed in aqueous extract.

The aqueous and ethanolic extracts of both plant materials were screened for their antimicrobial potential by standard disc diffusion technique against various clinical pathogens. Also the effect was assessed at three different levels of 2, 4, & 6mg of the extracts. The inhibitory effect produced by the extracts was compared with effect produced by the standard drug *Ciprofloxacin* 5µg and *Clotrimazole* 10µg was used as reference standard.

Table 1: Phytochemical investigation of *Aristolochia bracteata* extracts

S. No	Constituents	Aqueous	Ethanol	Method
1	Alkaloids	+	+	Mayers reagent, Hagers and Wagners reagent
2	Carbohydrates	-	-	Molisch's test
3	Glycosides	-	-	Borntrager's test
4	Phytosterols	+	+	Liebermann burchard
5	Saponins	+	+	Agitated with distilled water → foam
6	Fixed oils & fats	+	+	KOH + Phenolphthalein
7	Tannin & phenols	-	-	FeCl ₃ , Lead acetate
8	Protein & Amino acids	+	+	Millons reagent
9	Gums & mucilages	+	+	Alcohol
10	Flavonoids	-	+	Con. H ₂ SO ₄
11	Lignin	-	-	Phloroglucinol HCl

Table 2: Phytochemical investigation of *Cassia tora* extracts

S. No	Constituents	Aqueous	Ethanol	Method
1	Alkaloids	+	+	Drangondroffs reagent
2	Carbohydrates	+	+	Molisch's test
3	Glycosides	+	+	Borntrager's test
4	Phytosterols	+	+	Liebermann burchard
5	Saponins	-	-	Distilled water
6	Fixed oils & fats	+	+	KOH + Phenolphthalein
7	Tannin & phenols	-	-	FeCl ₃ , Lead acetate
8	Protein & Amino acids	-	-	Millons reagent
9	Gums & mucilages	+	-	Alcohol
10	Flavonoids	+	+	Con H ₂ SO ₄
11	Lignin	-	-	Phloroglucinol HCl

Table 3: Antimicrobial activity of extracts of *Aristolochia bracteata*

S. No	Test Organism	Diameter of zone of inhibition (mm)						
		Standard Drug	Aqueous Extracts			Ethanollic Extracts		
			S ₁ (2mg)	S ₂ (4mg)	S ₃ (6mg)	S ₁ (2mg)	S ₂ (4mg)	S ₃ (6mg)
1	Staphylococcus aureus	29	-	-	7	10	12	13
2	Staphylococcus albus	36	-	-	7	9	11	12
3	Streptococcus faecalis	37	-	-	-	9	11	12
4	Bacillus subtilis	25	-	-	8	8	11	13
5	Pseudomonas aerogenosa	32	-	-	6	7	8	12
6	Escherichia coli	27	-	-	7	11	11	21
7	Klebshiella aerogenes	22	-	-	6	12	12	15
8	Proteus vulgaris	28	-	-	-	13	14	16
9	Candida albicans	10	-	-	-	10	11	13
10	Aspergillus niger	11	-	-	-	13	14	14

Ciprofloxacin 5µg/disc for bacteria, *Clotrimazole* 10µg/disc for fungi, Solvent: Ethanol

Table 4: Antimicrobial activity of extracts of *Cassia tora*

S. No	Test Organism	Diameter of zone of inhibition						
		Standard Drug	Aqueous Extracts			Ethanollic Extracts		
			S ₁ (2mg)	S ₂ (4mg)	S ₃ (6mg)	S ₁ (2mg)	S ₂ (4mg)	S ₃ (6mg)
1	Staphylococcus aureus	31	-	-	8	11	11	18
2	Staphylococcus albus	36	-	-	7	10	13	14
3	Streptococcus faecalis	38	-	-	6	7	10	12
4	Bacillus subtilis	25	-	-	7	7	11	12
5	Pseudomonas aerogenosa	32	-	-	8	11	12	13
6	Escherichia coli	26	-	-	7	12	12	13
7	Klebshiella aerogenes	22	-	-	7	12	12	14
8	Proteus vulgaris	28	-	-	6	11	12	12
9	Candida albicans	10	-	-	-	8	9	10
10	Aspergillus niger	12	-	-	-	13	14	18

Ciprofloxacin 5 µg/disc for bacteria, *Clotrimazole* 10 µg/disc for fungi, Solvent: Ethanol

Table 3 & 4 showed the antimicrobial effect produced by the plant extracts against various pathogenic bacteria and fungi. This reveals that nil effect at 2 & 4mg levels and very minimum effect at 6mg level produced by the aqueous extracts of both the plants. In both ethanolic plant extracts showed increased inhibitory effect with respect to increase in level of drug concentration used against the test organisms.

The effect produced by ethanolic extract of *Aristolochia bracteata* (Table 3) was being maximum with gram negative bacteria [Escherichia coli (21mm), Kliebshiella aerogenes (15mm) and Proteus vulgaris (16mm)] than the other organisms under test. The effect produced against the fungi was being maximum as comparable with the effect of standard drug.

The ethanolic extract of *Cassia tora* (Table 4) showed higher inhibition against *Staphylococcus aureus* (18mm) and effect against other gram positive and gram negative bacteria under study is being moderate. The inhibition produced against fungi was found to be maximum as compared with the effect of standard drug.

CONCLUSION

We conclude that ethanolic extracts of *Aristolochia bracteata* and *Cassia tora* have remarkable antimicrobial activity. Aqueous extracts did not show significant antimicrobial activity against the microorganism under study

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