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EVALUATION OF ANTIMICROBIAL ACTIVITIES OF METHANOLIC EXTRACT AND FRACTIONS OF *FICUS HISPIDA LINN*. FRUITS

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ABSTRACT

The present study was carried out to investigate the methanolic extract of *Ficus hispida* (Bengali name- Kakdumur Family-Moraceae) fruits and it's fractions (chloroform, ethyl acetate and aqueous fractions) for their *in vitro* antimicrobial activities. The test was carried out against six pathogenic bacteria using agar disc diffusion method. The extract and it's fractions exhibited reasonable antibacterial activities against three gram positive (*Bacillus cereus*, *Staphylococcus aureus*, *Agrobacterium species*) and three gram negative (*Escherichia coli*, *Shigella dysenteriae*, *Shigella sonnei*) pathogenic bacteria. Ethyl acetate fraction (EAF) showed highest zone of inhibition (20.5 and 28 mm in diameter) against *E. coli* at a concentration of 200 and 400 µg/disc, respectively. The activity of crude methanolic extract (CME) was higher (20 nm in diameter) than EAF against gram positive bacteria. The chloroform fraction (CHF) did not show any activity against both gram positive and gram negative bacteria. Our present study suggests that active antimicrobial agents present in the extract of *Ficus hispida* fruits may have potential for the treatment of bacterial infection.

Key words: Antibacterial activity, *Ficus hispida*, Disc diffusion method.

INTRODUCTION

The problems of drug resistance, patient's sensitivity and inability to control certain infectious diseases have given an impetus for continuous search of new antibiotic all over the world and the treatment of infections is becoming difficult day by day. To combat the multi-drug resistant organisms, the screening of new antimicrobial compounds or antibiotics from new source is essential today (Aibinu *et al*., 2004; Aibinu *et al*., 2003a; Aibinu *et al*., 2003b). A number of phytochemical found in higher and lower plants have been known to have antimicrobial effect. In recent years, there has been a global trend towards the use of natural phytochemicals present in natural products such as fruits, vegetables, and their extracts (Ghadam AK *et al*., 2011).

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The World Health Organization (WHO, 2001) has estimated that between 80% of the populations of developing countries use traditional medicines-mostly plant drugs- almost consider them for primary healthcare needs. The major reason for this may be the high cost and unavailability of synthetic medicine. On the other hand the side effects of the synthetic agents may contribute to this.

Ficus hispida which is locally called as Kakdumur in Bangladesh belongs to the family Moraceae is a plant commonly grown in Australia, Malyasia and South East Asia including Bangladesh. The local people have been used the plant to treat some common infections such as ulcers, psoriasis, anemia, piles, jaundice, vitiligo, hemorrhage, diabetes, convulsion, hepatitis, dysentery, biliousness, and as lactagogue and purgative (Mohammad Ali *et al*., 2011) for thousands of years in Bangladesh specially the fruit. Literature survey revealed that alkaloids, carbohydrates, proteins and amino acids, sterols, phenols, flavonoids, gums and mucilage,

glycosides, saponins, and terpenes are major constituents in *Ficus hispida*. Successive extraction of methanolic extract of *Ficus hispida* fruits provided ethyl acetate, chloroform and Aqueous extracts.

Various scientific works like antineoplastic, cardioprotective, neuroprotective and anti-inflammatory effects (Pratumvinit *et al*., 2009; Yokota *et al*., 2006; Acharya *et al*., 1984; Vishnoi *et al*., 2004) have been published to establish the scientific basis of traditional medicinal values attributed to *Ficus hispida*. On the other hand, the n-hexane soluble fraction and methanol soluble fraction of ethanol extract of *Ficus hispida* revealed moderate antibacterial activity against some microorganisms (Shahriar *et al*., 2013). Till now, no another work has been published to elaborate the antimicrobial activities of the fruits of *Ficus hispida* Linn. This study is part of an ongoing project to search for novel drugs from a vast array of medicinal plants from the Northern part of Bangladesh. As part of our continuing studies of medicinal plants in Bangladesh the present study was, therefore, an effort to give a detailed account on its antimicrobial activities of the methanolic extract and the different fractions of *Ficus hispida* fruits.

MATERIALS AND METHODS

Collection and identification of the plant

The fruits of *Ficus hispida* were collected from Dinajpur, Bangladesh, in September July 2013. The plant was identified by the taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh and a voucher specimen was deposited in the herbarium unit.

Preparation, extraction and fractionation of plant material

The fruits were then sliced and air dried and powdered. The sun dried powdered fruits (500 gm) of *Ficus hispida* was macerated in 2.5 l of 99.8% methanol. After 15 days the solution was filtered using filter cloth and Whatman® filter paper No. 1. The resulting filtrates were then evaporated in a rotary evaporator at 40 °C and a brown semisolid mass (42 gm) of the extract was obtained. The concentrated methanolic extract was partitioned by modified Kupchan method and the resulting partitionates were ethyl acetate (2.5 gm), chloroform (4.5 gm) and aqueous fraction (1.8 gm) were used for antibacterial activities**.**

In Vitro Antimicrobial Screening

Disc Diffusion Method

Disc diffusion method was used to determine the antimicrobial activity of the crude extracts and it's fractions (Bauer *et al*., 1966) against the microbial strains gram positive bacteria then gram negative bacteria.Investigation of the antimicrobial activity of *Ficus hispida* has been investigated previously by listed in Table 1. These were collected as pure cultures from the Microbiology Laboratory of Rajshahi University, Bangladesh**.** Experimental sample was dissolved in methanol. Here, Standard disc of Kanamycin (30 µg/disc) and blank discs (immersed only in solvent) were used as positive and negative control, respectively. The sample disc was prepared by applying the desired concentration of extract on the sterilized filter paper disc in aseptic condition with a micropipette. The discs were then allowed to dry for few minutes to evaporate the solvent. Then, for 24 hours, these plates were kept at low temperature (4^{o}C) to allow maximum diffusion of the test materials and Kanamycin. To allow maximum growth of the organisms, these plates were then incubated for 24 hours, at 37.4^{o}C. The test materials, which possess antimicrobial activity, suppressed the growth of the microorganisms and a clear, distinct zone of inhibition was seen surrounding the discs. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition, expressed in mm.

Test microorganisms

Antibacterial activity was determined against three gram positive bacteria (*Staphylococcus aureus, Bacillus cereus, Agrobacterium species*) and three gram negative (*Escherichia coli, Shigella dysenteriae* and *Shigella sonnei*). All these organisms were collected from the Microbiology Research Laboratory of Pharmacy Department, University of Rajshahi, Rajshahi, Bangladesh.

RESULTS AND DISCUSSION

As a part of screening of the antimicrobial activities of *F. hispida* fruits, several fractions of extract were evaluated by zone of inhibition. After performing the zone of inhibition test, three different fractions (EAF, CHF and AQF) were found to have antimicrobial activity. The crude extract and its different partitionates when subjected to antimicrobial screening at 400 µg/disc and at 200 µg/disc, the CME and it's fractions CHF, EAF and AQF revealed antimicrobial activity against the tested microorganisms having the zone of inhibition ranging from 9 (*Agrobacterium species*) to 30mm (*Bacillus cereus*). Among them CME was found to have the highest inhibitory effect against the gram positive bacteria while AQF is most effective against gram negative bacteria. The CHF does not show any activity against both gram positive and gram negative bacteria at both concentrations. A considerable increase in inhibitory effect was found for increased concentration of sample. All the fractions show increase in activity at 400µg than at 200µg. Again the samples have higher activity against (Shahriar *et al*., 2013) and n-hexane soluble fraction and methanol soluble fraction of ethanol extract of *Ficus hispida* revealed moderate antibacterial activity against

some microorganisms (Shahriar *et al*., 2013). *Shigella dysenteniae* was the most susceptible isolate tested while *Agnobacterium species* was the least susceptible isolate. In this study it is observed that the potency of fruit is enhanced by the type of solvent used indicating that there are some active ingredients in fruits of *Ficus hispida* which have potent antimicrobial effect but which would not be released except when fruit is used in conjunction with a particular solvent.

Table 1. Zone of inhibition of the different fractions of *F. hispida* fruits against gram positive and gram negative bacteria

Types of organism	Name of organisms	Zone of inhibition in mm at 200 µg/disc					Zone of inhibition in mm at 400 µg/disc				
		CME	CHF	EAF	AQF	KAN	CME	CHF	EAF	AQF	KAN
Gram positive	*Bacillus cereus*	20	NA	19	16.5	38	27.5	NA	30	25	38
	Staphylococcus aureus	18.5	NA	16.5	14	36.5	26	NA	24	22	36
	Agrobacterium species	8.5	NA	9	11.5	38	14	NA	17	18	38
Gram negative	*Esherichia coli*	16.5	NA	20.5	18	39	18	NA	28	25	39
	Shigella dysenteniae	14	NA	15.5	19	34	18	NA	23.5	29	34
	Shigella soneii	11.5	NA	10	12	41	15.5	NA	13.5	17	41

CME = Crude methanolic extract, CHF = Chloroform fraction, EAF = Ethyl acetate fraction, AQF = Aqueous fraction, KAN = Kanamycin, NA = No activity.

CONCLUSION

It can be concluded that in this era of rapid antimicrobial drug resistance, the extracts of the *F. hispida* fruits can be used to design different new antimicrobial agents due to its high antimicrobial activities. *F. hispida* possesses various important pharmacological activities as discussed in the present review (Pratumvinit *et al*., 2009; Yokota *et al*., 2006, Acharya *et al*., 1984; Vishnoi *et al*., 2004). Further work is needed to isolate the secondary metabolites and the isolation of such bioactive components could perhaps clarify the pharmacological properties of *F. hispida* fruits and be further exploited for pharmaceutical use.

Additionally it is imperative that more pre-clinical and clinical studies along with establishment of better quality control methods should be conducted to elucidate the unexplored potential of fruits of this plant. Hence, there is a need to isolate possibly by purification of the various phytochemical groups in the extracts and it's fractions.

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REFERENCES

Acharya BM, Kumar KA. Chemical examination of the bark of *Ficus hispida* Linn. *Current Sci,* 53, 1984, 1034-5.

Aibinu I, Adenipekun E, Odugbemi T. Emergence of Quinolone Resistance amongst *Escherichia coli* strains isolated from clinical infections in some Lagos State Hospitals in Nigeria. *Nig J Hlth Biomed Sci*, 3(2), 2004, 73–78.

Aibinu I, Odugbemi T, Mee BJ. Extended-Spectrum Beta-Lactamases in Isolates of *Klebsiella* spp and *Escherichia coli* from Lagos, Nigeria. *Nig J Hlth Biomed Sci*, 2(2), 2003, 53–60.

Aibinu IE, Ohaegbulam VC, Adenipekun EA, Ogunsola FT, Odugbemi TO, Mee BJ. Extended-Spectrum Beta-Lactamase enzymes in clinical isolates of *Enterobacter* species from Lagos, Nigeria. *J Clinical Microbiol,* 41(5), 2003, 2197–2200.

Bauer AW, Kirby WMM, and Tuck M. Antibiotic susceptibility testing by a standardized disc diffusion method. *J. Am. Clin. Pathol,* 45, 1966, 493-496.

Ghadam AK, Nasrollah G, Farshid H and Marzeih S. *Iran J Pharm Res*, 10(2), 2011, 273–277.

Mohammad A and Nisha C. *Ficus hispida* Linn, A review of its Pharmacognostic and Ethnomedicinal properties. *Pharmacognosy Rev*. 5(9), 2011, 96-102.

Pratumvinit B, Srisapoomi T, Worawattananon P, Opartkiattikul N, Jiratchariyakul W, Kummalue T. *In vitro* antineoplastic effect of *Ficus hispida* Plant against breast cancer cell lines. *J Med Plants Res*, 3, 2009, 255-261.

Prescott ML, John P, Harley D, Klein A. Microbiology.2nd ed. C. Brown publishers. *Antimicrobial Chemotherapy*, 1999, 328.

Roland R. Antibiotics, An Introduction. Switzerland, F. Hoffmann La Roche and Co, Basel, 1982.

Shahidi Bonjar GH. Evaluation of antimicrobial properties of Iranian medicinal plants against *Micrococcus luteus, Serratiamarcescens, Klebsiella pneumoniae* and *Bordetella bronchoseptica*. *Asian J Plant Sci*, 3(1), 2004, 82–86.

Shahriar M, Islam MS, Parvin, S, Hoque S. Thrombolytic activity and antimicrobial properties of *Ficus hispida*. *Journal of Scientific Research*, 5(2), 2013, 393-397.

The World Health Organization "Traditional'' Fact Sheet number 134, 2003.

VanWagenen BC, Larsen R, Cardellina JH, Ran DD, Lidert ZC, and Swithenbank C. Ulosantoin, a potent insecticide from the sponge *Ulosaruetzleri*. *J Org Chem*, 58, 1993, 335-337.

Vishnoi SP, Jha T. Evaluation of anti-inflammatory activity of leaf extracts of *Ficus hispida*. *Indian J Nat Prod*, 20, 2004, 27-29.

Yokota JD, Takuma A, Hamada M, Onogawa S, Yoshioka, Nishioka Y. Scavenging of reactive oxygen species by *Eriobotrya japonica* seed extract. *Biol Pharm Bull*, 29, 2006, 467-71.