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Full Length Research Paper

In-vitro membrane stabilizing, thrombolytic, antioxidant and antimicrobial activities of Bangladeshi origin *Coccinia indica* (Cucurbitaceae)

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This study was made to investigate *in-vitro* membrane stabilizing, thrombolytic, antioxidant and antimicrobial activities of ethanolic leaf extract of *Coccinia indica*. Different extractives showed significant membrane stabilizing activity. The ethanol soluble fraction showed (45.36±0.45 % and 64.05±0.22 %) of Red blood cells haemolysis inhibition under heat and hypotonic solution induced conditions, respectively. Mention worthy that carbon tetrachloride soluble fraction of *C. indica* exhibited highest thrombolytic activity 57.94±0.23% in a comparison with standard streptokinase (SK) (68.89±0.35 %). The antioxidant activities were determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging. In this case, ethanol soluble fraction (ESF) of crude extract showed fairly significant scavenging (with IC50 value of 2.52 µg/ml) as opposed to a well-known antioxidant, butylated hydroxytoluene (BHT) (IC50 value of 3.99 µg/ml). Pet ether soluble fraction (PESF) and ESF showed mild inhibitory effects against some Gram-positive and Gram-negative bacteria (zone of inhibition was 12 and 13 mm). The overall findings of the studies demonstrated that the leaves of *C. indica* has fairly significant membrane stabilizing and thrombolytic activity, mild to moderate antioxidant and antimicrobial potential. Thus, may indicated the scientific basis of *C. indica* leaves as a remedy for traditional uses against fever, pain, oxidative stress, inflammation, infectious diseases, diabetes, etc.

Key words: *Coccinia indica*, *in vitro*, thrombolytic, 1,1-diphenyl-2-picrylhydrazyl, antimicrobial activities.

INTRODUCTION

Coccinia indica is botanically known as Ivy Gourd (Family: Cucurbitaceae) and traditionally familiar as “Telakucha”. This opportunistic plant is used as wild vegetable in several areas of Asia specially in cooking and as medicines (Sadique et al., 1989). It assumed to have various significant medicinal properties like analgesic, antipyretic, anti-inflammatory, antimicrobial, antiulcer, antidiabetic, antioxidant, hypoglycemic, hepatoprotective, antimalarial, antidyslipidemic, anticancer, antitussive and mutagenic properties, etc. (Rahmatullah et al., 2009; Priyanka et al., 2016). It is an annual creeper having tuberous roots, grown over ground

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Table 1. Results of different chemical groups of *C. indica* extract.

Test	Result
Alkaloids	+ ve
Flavanoids	+ve
Terpenoids	-ve
Saponins	-ve
Tannins	+ve
Phenols	+ve
Resin	+ve
Coumarin	+ve
Glycosides	+ve

and twilling around the trees. The stem is pentagonal, leaves are triangular or pentagonal, flowers are white color, monoicous, fruits are slimy, pulpy and barrel shaped containing seeds inside (Deokate et al., 2012).

The methenolic extract of *C. indica* fruit contains alkaloids, steroids, tannins, saponins, ellagic acid, phenols, glycosides, lignans and triterpenoids. Roots containes Triterpenoid, saponincoccinioside, Flavonoid glycosideombuin 3-O-arabinofuranoside, Lupeol, β-amyrin, β- sitosterol and Stigmast-7-en-3-one. *C. indica* contains various phytoconstituents like lupeol, β-amyrin, cucurbitacins, taraxerol, β-carotene, steroidalsaponins, flavonoids, pectin and polyprenol. Phytoconstituents such as momordicin and cucurbitanes reported earlier from the plants and considered for its antidiabetic activity (Laboni et al., 2017) since no literature is currently available to claim this activity. The conducted study involved the evaluation of beneficial effects of *C. indica* of Bangladeshi origin to substantiate *in-vitro* membrane stabilizing, thrombolytic, antioxidant and antimicrobial properties. Also to provide scientific evidence for its use as a traditional folk remedy by investigating the above pharmacological potential that will confirm its use in folk remedy for inflammation, pain and other pathological disorders implicated with free radicals.

MATERIALS AND METHODS

Collection and identification

The leaves of *C. indica* were collected from Dhaka, Bangladesh during February, 2017 at flowering stage and the plant samples were identified and authenticated by Bangladesh National Herbarium Mirpur, Dhaka with an Accession No: DACB 45113 for further reference. The leaves were made contaminant free.

Ethanolic extract

The collected plant materials freed from undesirable materials were sun dried for 7 days and then pulverized. Then about 250 g of powdered materials collected were soaked in 1600 ml of ethanol at room temperature for 10 days. The extracts were filtered through fresh cotton bed and finally with Whatman filter paper number 1 and concentrated with a rotary evaporator at reduced temperature and pressure. It is concentrated 33 g of gummy materials (13.20%) that was designated as crude ethanol extract.

An aliquot (5 g) of each of the concentrated ethanol extract was fractionated by the modified Kupchan partition protocol and the resultant partitionates were further evaporated to dryness with rotary evaporator to yield ethyl soluble fraction (ESF), hexane soluble fraction (HXSF), carbon tetrachloride soluble fraction (CTSF), aqueous soluble fraction (AQF). Fractionates were stored in a refrigerator for further use.

Different chemical groups

The ethanolic extract was then screened for its different chemical groups like alkaloids, flavonoids, gums, reducing sugars, saponins, steroids and tannins (Table 1) (Amir et. al., 2014; Evans, 1989).

Membrane stabilizing activity

The method developed by Omale et al., 2008 was used to evaluate the membrane stabilizing activity of the extractives. The inhibitory capability of hypotonic solution and heat-induced haemolysis of human erythrocytes were evaluated (Sadique et al., 1989).

Thrombolytic activity

Following the thrombolytic activity, it was evaluated by using streptokinase (SK) as positive control (Prasad et al., 2007).

Antioxidant activities

According to (Chang et al., 2001) was implied for performing the scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical by synthetic antioxidants, butylated hydroxytoluene (BHT) as positive controls to assess the antioxidant activity of the plant extracts and its partitionates (Brand et al., 1995).

Antimicrobial screening

This was determined by using a modified Kirby-Bauer disc diffusion method (Bauer et. al., 1966).

Statistical analysis

For all bioassays, three replicates of each sample were used for statistical analysis and the values are reported as mean ±SD (Mita et. al., 2017).

Human and animal rights

This study does not contain any studies performed with human and animal subjects.

RESULTS

Phytochemical constituents

The crude ethanolic extract and its different fractions

Table 2. Effect of different *C. indica* (leaves) on hypotonic solution and heat induced RBCs haemolysis of erythrocyte.

Fraction	Haemolysis inhibition (%)	
	Heat induced	Hypotonic solution induced
ESF	45.36 ± 0.45	64.05±0.22
PESF	41.08 ±0.34	50.19±0.52
HXSF	28.26 ±0.11	65.75±0.88
CTSF	37.65 ±0.34	54.33±0.55
AQF	34.51 ±0.10	59.23±0.25
ASA	59.82 ±0.22	71.15±0.92

±SD (standard deviation). ESF: Ethyl soluble fraction; HXSF: hexane soluble fraction; CTSF: carbon tetrachloride soluble fraction; AQF: aqueous soluble fraction; ASA: acetyl acetic acid.

Table 3. The percent clot lysis by different *C. indica* extracts.

Fraction	Weight of empty vial (w1, g)	Weight of vial with clot (w2, g)	Weight of vial with clot after lysis (w3, g)	Weight of clot lysis (w2-w3, g)	Weight of clot before lysis (w2-w1, g)	Clot lysis (%)
ESF	6.17	6.70	6.49	0.22	0.53	40.89 ± 0.71
PSF	6.15	6.54	6.33	0.21	0.39	54.64 ± 0.26
HXSF	6.21	6.71	6.59	0.13	0.50	25.09 ± 0.04
CTSF	6.70	6.94	6.80	0.14	0.24	57.94 ± 0.23
AQSF	6.16	6.66	6.42	0.23	0.50	46.87 ± 0.83
SK	4.64	5.09	4.78	0.31	0.45	68.89 ± 0.35
Blank	3.04	3.87	2.98	0.89	0.83	5.74 ± 0.36

W1: Weight of micro centrifuge tube alone; W2: weight of clot containing tube; W3: weight of clot containing tube after clot disruption; SK: Streptokinase.

showed presence alkaloids, flavonoids, tannins, phenols, resins, glycosides and coumarins (Table 1).

Membrane stabilizing activity

The extractives at a concentration of 1.0 mg/ml, prevent the haemolysis of RBC induced by heat and hypotonic solution as compared to the standard acetyl salicylic acid (0.10 mg/mL). The ethanolic soluble fraction inhibited (45.36±0.45 % and 64.05±0.22 %) haemolysis of RBCs as compared to (59.82±0.22 % and 71.15±0.92 %) by acetyl salicylic acid, respectively (Table 2).

Thrombolytic activity

The extract was assessed for thrombolytic activity (Table 3). 100 µl of SK (30,000 I.U.) was added to the clots as a positive control and incubated for 90 minutes at 37°C subsequently which showed (68.89±0.35**%**) lysis of clot. Negative control exhibited a negligible percentage of lysis of clot (5.74±0.36 %). Among other constituents, the ethanolic soluble fraction showed thrombolytic activity of (40.89±0.71), pet-ether soluble fraction (54.64±0.26 %), hexane soluble fraction (25.09±0.04 %), carbon tetrachloride soluble fraction (57.94±0.23%) and aqueous soluble fraction showed (46.87±0.83 %) lysis.

Antioxidant activities

IC50 values of ascorbic acid was 3.99 µg/ml whereas the extractives from *C. indica* were found (1.45, 2.52, 6.55, 4.65 and 10.68 µg/ml) for (ESF, PESF, HXSF, CTSF and AQSF), respectively. ESF of crude extract showed highest scavenging with IC50 value of 2.52 µg/ml as opposed IC50 value of well-known antioxidant BHT (3.99 µg/ml). IC50 values of ethanolic extract and its partitionates have been presented (Figure 1).

Antimicrobial activity

The extractives showed mild antimicrobial activity against some Gram-positive *(Bacillus cereus, Staphylococcus aureus, Bacillus subtilis, Sarcina lutea) and Gram-*negative *(Salmonella typhi, Vibrio parahemolyticus E.*

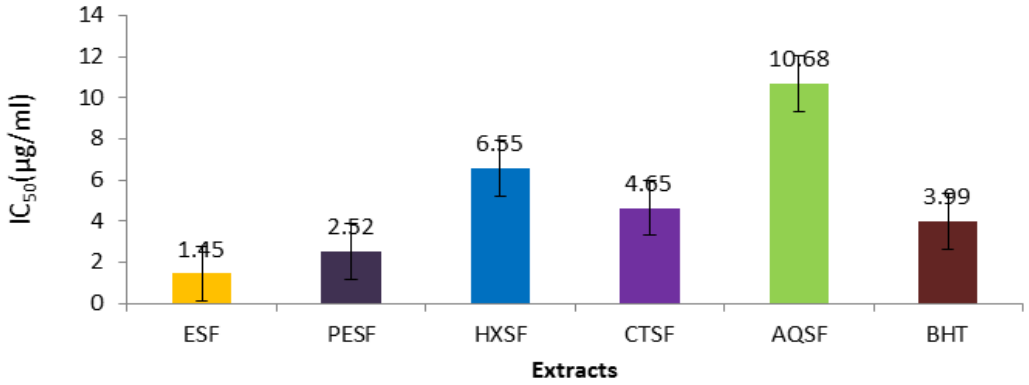

Figure 1. IC50 values of the standard BHT and different *C indica* extracts. BHT: Butylated hydroxyl toluene, ESF: ethanolic soluble fraction, HXSF: hexane soluble fraction, PESF: pet-ether soluble fraction, CTSF: carbon tetrachloride fraction, AQSF: aqueous Soluble fraction.

Table 4. Antimicrobial activity of *C indica different* fractionates and ciprofloxacin.

Test organism	Inhibition of Zone Diameter (mm)					
	ESF	PESF	HXSF	CTSF	AQSF	CpF
Gram-positive bacteria						
B. cereus	7.0	9.0	12.0	7.0	6.0	4.3
S. aureus	9.0	7.0	11.0	5.0	6.0	4.5
B. subtilis	9.0	6.0	10.0	4.0	7.0	4.7
S. lutea	10.0	10.0	9.0	7.0	10.0	4.4
Gram-negative bacteria						
S. typhi	11.0	7.0	9.0	10.0	8.0	4.7
V. parahemolyticus	8.0	11.0	6.0	9.0	7.0	4.2
E. coli	6.0	5.0	7.0	5.0	6.0	4.7
V. mimicus	7.0	12.0	8.0	9.0	5.0	4.5
S. dysenteriae	11.0	13.0	10.0	8.0	7.0	4.3

All the values were taken thrice times and their average values have been given in the table. ESE: Ethanol soluble fraction, PESF: pet ether soluble fraction, HXSF: hexane soluble fraction, CTF: carbon tetrachloride soluble fraction, AQSF: aqueous soluble fraction, CpF: ciprofloxacin.

coli, Vibrio mimicus, Shigella dysenteriae) bacteria. The inhibition zone was found (5 to13 mm) by 200 µg of each extractives (Table 4).

DISCUSSION

It reported that flavonoids exert profound stabilizing effects on lysosomes both *in-vitro* and *in-vivo* in experimental animals while tannin and saponins had ability to bind cations and other biomolecules, and were able to stabilize the erythrocyte membrane (Oyedapo et al., 2004). The high membrane stabilizing activity of *C. indica* (leaves) extracts observed due to the presence of tanins and flavonoids. Several investigations reported that phytoconstituents were capable exerting anti-inflammatory activity by stabilizing red blood cell membrane (Olugbenga et al., 2005; Shinde et al., 1999). Alkaloids, flavonoids, tannins and terpenoids were responsible for thrombolytic activity (Tsuchida et al., 2005). In search of natural cardio protective agents, *C. indica* leaves assessed pet-ether soluble fraction showed highest activity for presence of abovementioned phytoconstituents. However, many sophisticated and modern technologies are now available for finding out mechanism by applying agents, this reliable and more practiced simplified model of clot lysis may be the primarily initiative.

DPPH free radical scavenging ensured that the plant extracts could donate hydrogen proton to the lone pair electron of the radicals. Tannins and certain flavonoids established free-radical scavengers innately linked to

their chemical structure (Beaudelaire et al., 2010; Takako et al., 1998). Due to presence of various phytochemical components, especially polyphenols like flavonoids, tannins etc. were thought responsible for the free radical scavenging and antioxidant activities of *C. indica*. The scientists are still in search of investigating antimicrobial activity of different medicinal plants as natural reservoir of many antimicrobial agents to combat microbial resistance (Austin et al., 1999). The ethanolic (ESF) and pet-ether soluble fraction (PESF) showed mild inhibitory effects against some broad-spectrum Gram-positive and Gram-negative strain of bacteria. Though their antimicrobial actions are not so prominent, isolation of pure phytoconstituents from this soluble fraction will lead to development of novel antimicrobial agents.

CONCLUSION

The investigation showed promising *in-vitro* membrane stabilizing thrombolytic, antioxidant and antimicrobial properties of the leaves of *C. indica.* This might be a scientific basis of the proven treatment for use of *C. indica* in various ailments. Along with this preliminary study, the extracts must check for phytochemical and pharmacological activity to find out more specific medicinal and pharmaceuticals potentials. Thus, the specific active ingredients responsible for the presented activities will be able to isolate and characterize to establish the mechanism of action.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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