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EVALUATION OF ANTIBACTERIAL ACTIVITY OF *HIBISCUS SABDARIFFA* LEAF EXTRACT USING DISK DIFFUSION METHOD

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ABSTRACT

Hibiscus sabdariffa L. is widely used in the preparation of beverages. Active component of this plant exhibit antimicrobial activity, yet little research has been conducted on their possible use in food systems as antimicrobials. The use of this traditional medicinal plant as natural antimicrobial agents is gaining popularity. The *Hibiscus sabdariffa* is widely used for the treatment of diseases. The present study is the continuation of a program aimed at investigation of antimicrobial properties of *Hibiscus sabdariffa* leaf extract. The antibacterial activity was evaluated according to the disk diffusion method by using Gram positive; *B. subtilis*, *S. aureus* and *Enterococcus* and Gram negative; *E. coli*, *Acetobacter*, *Citrobacter*, bacteria. This study shows that methenolic leaves extracts of *Hibiscus sabdariffa* Linn inhibit the growth of microorganism's dose dependently. The appear results confirm that the antibacterial activity of *Hibiscus sabdariffa* leaves.

KEYWORDS

Antibacterial, Disk Diffusion method, Bacteria and *Hibiscus sabdariffa*.

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INTRODUCTION

Hibiscus sabdariffa (Roselle, Ambadi bhaji), a member of the Malvaceae family, is a species of *Hibiscus* native to West Africa, middle East and Indian subtropical. In Indian, Ayurveda medicine has used many herbs such as turmeric possibly as early as 1900 BC (Aggarwal *et al*, 2007)¹. The traditional literatures such as the Rig Veda, and Atharva Veda are some of the earliest available documents detailing the medical knowledge that formed the basis of the Ayurveda system (Sumner and Judith, 2000)². Some important medicinal plants and minerals used in Ayurveda were later

described by ancient Indian herbalist. Many research was carried out on *Hibiscus sabdariffa* L. family Malvaceae calyx ethanol extract in order to ascertain its physiological activity-structure relationship are generally located in areas where it is used in food applications and traditional medicine (Ross, 2003)³. The therapeutic effects of *H. sabdariffa* on lipid metabolism was observed (El-Saadany, et al, 1991⁴, Wang, et al, 2000⁵, Chen, et al, 2003⁶, Carvajal-Zarrabal, et al, 2005⁷, Hirunpanich, et al, 2006⁸, Lin, et al, 2007⁹). Antihypertensive effects was discovered (Faraji, and Tarkhani, 1999¹⁰, Onyenekwe, et al, 1999¹¹, Odigie, et al, 2003¹², Herrera-Arellano, et al, 2004¹³, Ajay, et al, 2007¹⁴). The Apoptotic effects of this plant in gastric carcinoma cells was also showed (Chang, et al, 2006¹⁵, Lin, et al, 2007¹⁶). *H. sabdariffa* L. leaf has also been subjected to this type of scientific studies, particularly leaf ethanolic extracts, which have been found to influence lipid metabolism (Essa, et al, 2009)¹⁷. *Hibiscus sabdariffa* calyx extract contains hibiscus acid, or (+)-hydroxycitric acid, known as (+)-HCA (Ibnusaud and Thomas, 2003¹⁸, Jena, et al, 2002)¹⁹ which was useful in several diseases. It has been proposed as an anti-obesity agent (Sullivan, et al, 1974²⁰, Heymsfield, et al, 1998²¹, Hayamizu, et al, 2003)²². The powder made of dried crushed leaves is called ambadi bhaji. The antioxidants including flavonoids, gossypetine, hibiscetine and sabdaretinewas present in plant extract. This plant extract was also rich in riboflavin, ascorbic acid, niacin, carotene, calcium, and iron that are nutritionally important. *Hibiscus sabdariffa* L (HS) (*Malvaceae*), a common local drink popularly known as *zobo* in Nigeria and medicinal herb, is used in folk medicine in the treatment of hypertension (Wang et al, 2000⁵, Odigie et al, 2003¹², Olaleye and Akindahunsi, 2005)²³. Therefore, we have planned out the antibacterial activity of *Hibiscus sabdariffa* leaf extract on different type of bacteria.

MATERIAL AND METHODS

Plant Material

The *Hibiscus sabdariffa* (*Ambadi bhaji*) leaves were collected from local herbal garden of Raipur, Chhattisgarh and dried for few days in shade, which were then powdered and preserved in airtight bottles for further studies.

Extract Preparation

Hibiscus sabdariffa L. Leaves (20g) was extracted in 50% of methanol and Millipore water solvent the supernatant was collected and concentrated in water bath at 40-50 C .The dried powder was kept in air tied box.

Microorganisms

The tested microorganisms included the Gram positive bacteria; *Bacillus subtilis*, *Staphylococcus aureus*, (ATCC 25923) *Enterococcus* and Gram negative bacteria; *Acetobacter*, *Citrobacter*, *Escherichia coli*. These bacteria's strains were procured from National Chemical Laboratory (NCL), Pune, India. The bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C.

Antibacterial Assay

Antibacterial activity of *Hibiscus sabdariffa* L. Leaf extract was determined by agar disk diffusion method [Nair, et al, 2005]²⁴ at four concentrations i.e., 100, 75, 50 and 25mg/ml. Muller Hinton agar was prepared according to the manufacturer's instructions and the plates were seeded with appropriate microorganisms (*Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus*, *Acetobacter*, *Citrobacter* and *Escherichia coli*). Discs of 6 mm diameter were prepared from Whatmann filter paper No. 24 and sterilized. The discs were then impregnated with the extracts and solvent DMSO. Antibiotics for Gram positive (NX – Norfloxacin, OF- Ofloxacin, E-Erythromycin, CFM- Cefixime) and Gram Negative (NX–Norfloxacin, OF-Ofloxacin, E-Erythromycin, CFM- Cefixime). Bacteria were used as standard. The plates were incubate at 37°C for 24 hrs and the zones of inhibition were measured with a measuring scale. Above experiment was carried out in triplicate for their confirmation.

RESULTS AND DISCUSSION

The results showed that the extracts of *Hibiscus sabdariffa* L. has a concentration dependent antibacterial activity with more sensitivity for gram positive bacteria than gram negative bacteria used in the extracts of *Hibiscus sabdariffa* showed considerable antibacterial activity at all the four concentrations 100, 75, 50, 25 mg/ml (Table No.1). (Table No.2) shows the sensitivity of the tested bacteria to the standard antibiotics.

The medicinal value of plants lies in some chemical substances that have a definite physiological function in the human body. Some different phytochemicals have been found to possess a wide range of medicinal properties, which may help in protection against various diseases. Mainly, alkaloids protect against chronic diseases; saponins protect against hypercholesterolemia and steroids and triterpenoides show the analgesic properties (Geetha et al, 2014)²⁵. Some previous findings revealed that these plant present phenolic compounds including flavonoids are potent antioxidants with reported antimutagenic and anticarcinogenic effects. Tannin contributes various medicinal properties such as antimicrobial, anti-inflammatory and astringent activity (Geetha et al, 2014)²⁵.

Through the ages man has learnt to take advantage of the many resources placed at his disposal by nature to meet his essential needs in all fields. As important reserves and sources of abundance, natural resources are indispensable for socio-economic development. Many plants of the Malvaceae family have been found to possess antimicrobial properties against several microorganism of clinical importance. In this study, we reported the antibacterial effect of *Hibiscus sabdariffa* extract.

Our result for antibacterial activity of plant *Hibiscus sabdariffa* shows that antibacterial activity of 50% Methanolic extract of *Hibiscus sabdariffa* against different selected bacteria. The above observations suggests that different concentration (25%, 50%, 75%, 100%) were having good antibacterial activity against Acetobacter,

Citrobacter, E.coli, Bacilli Subtilis, Staphylococcus aureus and Enterococcus. Thus the extract is showed varying activity against all organisms. On comparing the zone of inhibition of extract to that of standard antibiotics extract showed better activity than Ciprofloxacin (CIP), Doripenem (DOR), Ofloxacin (OF), Maxifloxacin (OM) in these conditions. The development of antibiotic resistance has become a global public health challenge which is causing in effectiveness of anti-bacterial agents leading to increase in diseases and death rate. Therefore this study intends to investigate the antibacterial action of *Hibiscus sabdariffa* (Ambadi bhaji) extract against multidrug pathogens.

The antibacterial activity obtained in this study, coincides with those reported by other researchers (Mei-Chi and Che-Yi, 2008)²⁶. The antibacterial activity of selected plant may be attributed to the phytochemical compounds it contains especially those of phenolic nature such as gallic acid, quercetin, rutin and luteolin and analogues which were detected therein as previously reported in literature (El-kamali and Moneer, 2006²⁷, Alaa, 2012)²⁸.

The antibacterial activity of *Hibiscus sabdariffa* calyx extract provide valuable information and highlight the potentiality of this plant in drug development as a candidate source of antibacterial agents being safe and edible. Some more investigation is required to ascertain that the in vitro results are attainable *in vivo*. Moreover, this study strongly supports the ethno medical uses of *Hibiscus sabdariffa* calyx in treatment of cough, abscess and bilious conditions which are caused mainly by microbial infections (Morton, 1987)²⁹. It can be reported that *Hibiscus sabdariffa* calyx could be a potential source of antibacterial agents so further studies should be conducted to identify the active constituents responsible for this activity.

SUMMARY

Hibiscus sabdariffa is an ideal crop for developing countries as it is relatively easy to grow, can be grown as part of multi-cropping systems and can be used as food and fiber. In China the seeds are used

for their oil and the plant is used for its medicinal properties, while in West Africa the leaves and powdered seeds are used in meals. It is also used in the pharmaceutical and food industries. Our observations suggest that different concentration were having good antibacterial activity against different bacteria's. Thus the extract is showed varying activity against all organisms.

Experimental data suggested that antibacterial study was done which shows a better antibacterial activity against all the six test gram-positive and gram-negative bacteria species used and shown antibacterial susceptibility to extracts with clear zone of inhibition.

So, in future it can be used as an alternate to synthetic antibiotics. Much effort has needed to increase plant extract as a dietary supplement in food to resist the human pathogenic bacterial disease. This work has revealed further potentials of this plant in the area antimicrobial agent. As a result of the high antimicrobial activity, the extract of *Hibiscus sabdariffa* would be considered a safe antimicrobial agent.

Table No.1: Antibacterial activity of *Hibiscus sabdariffa* Leaves extract against tested bacteria

S.No	Test sample concentration in (mg/ml)	Name of the Microorganism (Inhibition Zone in mm)					
		Gram Positive			Gram Negative		
		<i>Bacillus subtilis</i>	<i>S.aures</i>	<i>Enterococcus</i>	<i>Acenetobacter</i>	<i>Citrobacter</i>	<i>E.coli</i>
1	100%	10	13	14	10	0.6	13
2	75%	0.8	15	11	0.9	0.0	14
3	50%	10	16	12	11	0.8	12
4	25%	11	12	11	0.8	0.8	10

Table No.2: The study of anti-bacterial activities of standard antibiotics using disk diffusion method

S.No	Name of Bacteria	Zone of Inhibition (In MM)			
		NX	OF	E	CFM
1	Gram Positive (+)				
	<i>Bacillus subtilis</i>	37.00	32.00	15.00	28.00
	<i>S. aureus</i>	34.00	31.00	15.00	09.00
	<i>Enterococcus</i>	39.00	36.00	20.00	35.00
2	Gram Negative (-)				
	<i>Acenetobacter</i>	15.00	09.00	09.00	09.00
	<i>Citrobacter</i>	37.00	30.00	15.00	28.00
	<i>E.coli</i>	34.00	30.00	18.00	21.00

CONCLUSION

Hibiscus sabdariffa is an ideal crop for developing countries as it is relatively easy to grow, can be grown as part of multi-cropping systems and can be used as food and fiber. In China the seeds are used for their oil and the plant is used for its medicinal properties, while in West Africa the leaves and powdered seeds are used in meals. It is also used in the pharmaceutical and food industries. Our

observations suggest that different concentration were having good antibacterial activity against different bacteria's. Thus the extract is showed varying activity against all organisms.

Experimental data suggested that antibacterial study was done which shows a better antibacterial activity against all the six test gram-positive and gram-negative bacteria species used and shown antibacterial susceptibility to extracts with clear

zone of inhibition. These potent antibacterial properties of the calyces of *H. sabdariffa* could be attributed to its richness in secondary phytochemical metabolites. The previous study on Sudanese roselle revealed the presence of alkaloids, phenolic compounds, flavonoids and saponins. These compounds are considered to be the major groups of antimicrobial compounds in plants.

So, in future it can be used as an alternate to synthetic antibiotics. Much effort has needed to increase plant extract as a dietary supplement in food to resist the human pathogenic bacterial disease. This work has revealed further potentials of this plant in the area antimicrobial agent. As a result of the high antimicrobial activity, the extract of *Hibiscus sabdariffa* would be considered a safe antimicrobial agent.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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