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EFFECT OF SOLVENT, TEMPERATURE AND TIME ON EXTRACTION PROCESS OF ANTI MICROBIAL FACTOR OF *STAPHYLOCOCCUS AUREUS* PRESENT IN *AMARANTHUS SPINOSUS L.*.

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ABSTRACT

Effect of solvent, temperature and time on extraction process of anti microbial factor against *Staphylococcus aureus* present in the leaves of *Amaranthus spinosus L.* was studied. Result showed that extraction of leaves of *Amaranthus spinosus L.* with methanol (50%, v/v) at 40^0C for one hour had maximum antibacterial effect against *Staphylococcus aureus.* Antibacterial effect was checked by disc diffusion method.

Key Words:- Extraction process, Antibacterial effect, *Staphylococcus aureus, Amaranthus spinosus L*.

INTRODUCTION

Multiple antibiotic resistance in bacterial populations is a pervasive and growing clinical problem, which is recognized as a threat to public health.Various bacteria like *Staphylococcus aureus*, *Pseudomonas aeruginosa* etc. are inherently resistant to many antimicrobial agents, mainly due to the energy between multi-drug efflux system or a type 1 AmpC beta lactamase and low outer membrane permeability (Hancock, 1998; Livermore, 2001; Coates et al., 2002; Chopra, 2003; Das RN *et al*., 2006). There is thus continuous effort for synthesis of new chemicals having antimicrobial activity (Mamalis, 1971; Joshi N *et al*., 1994; Baregama L. and Talesara GL, 2002). But most of these chemicals are potentially toxic and are not free of side effects on the host (Geddes, 1985).

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This has urged microbiologist for formulation of new antimicrobial agents and evaluation of the efficacy of natural plant products as the substitute for chemical antimicrobial agents (Pandian MR et al., 2006). In this context several plants were screened to know their antimicrobial property (Venugopal PV and Venugopal TV, 1994; Chakraborty et al., 1995; Padmaja V et al., 1995; Gopalakrishnan G et al., 1997; Rana BK et al., 1997; Suresh B et al., 1997; Valsaraj R et al., 1997).

In our laboratory we also screened many medicinal plants for their antimicrobial property and found the anti microbial role of the leaves of *Cassia alata* Linn against *Staphylococcus aureus* (Paul B et al., 2012). We also noted *In vitro* antibacterial activity of leaves of *Amaranthus spinosus* L. (Mitra Prasanta Kumar, 2014).

It was, therefore, felt to start isolation work of the antimicrobial compound present in *Amaranthus spinosus* L. In this communication effect of solvent, temperature and time on extraction process of anti microbial factor

against *Staphylococcus aureus* present in the leaves of *Amaranthus spinosus* L. is being reported.

MATERIALS AND METHODS

Plant material

Leaves of *Amaranthus spinosus* L. were collected from the medicinal plants garden of the University of North Bengal and authenticated by the experts of the department of Botany of the said University. A voucher specimen was kept in the department for future reference.

Preparation of leaves for Anti bacterial screening

Leaves of *Amaranthus spinosus* L.were shade dried and powdered. 100 grams of this powder was separately extracted with 500ml of different solvents at different temperatures and time on a rotary shaker. The extract was filtered and the solvent was evaporated to dryness *in vacuo* with rotary evaporator at $40 - 50\ ^0$C. A brownish mass was obtained. 500 micro gram of the mass is extracted with 1 ml water and the solution obtained there from was used to evaluate the anti bacterial activity against *Staphylococcus aureus*.

Effect of solvents in extraction process

Water as well as 50% (v/v) of acetone, ethanol, methanol and petroleum ether were used separately in extraction process.

Effect of time in extraction process

Extraction processes were done separately for 30, 60, 90 and 120 minutes.

Effect of temperature in extraction process

In separate experiment extraction processes were done at 30, 40 and 50 degree centigrade.

Reagents

All reagents required for the experiment were procured from Merck, USA.

Bacteria

Staphylococcus aureus used in his study was collected from the department of Microbiology, North Bengal Medical College Hospital.

Media

Nutrient agar media (Difco laboratories) pH 7.2 was used for antibacterial screening.

Antibacterial screening

In vitro antibacterial screening was carried out by disc diffusion method (Rahman MM et al., 2000). According to this method. 20 ml quantities of nutrient agar were placed in a petri dish with 0.1 ml of 10^{-2} dilution of bacterial culture of 20 hours old.

Filter paper discs (6 mm diameter) impregnated with 30 µg/per disc and 60 µg per disc concentration of the solution prepared from Leaves of *Amaranthus spinosus* L. were placed on test bacteria-seeded plates. Blank disc impregnated with water was used as negative control.
Zone of inhibition was recorded after 18 hours of incubation.

Each sample was used for five times for the determination of anti bacterial activity.

Statistical analysis

The values were expressed as mean ± SEM and were analyzed using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) 20th versions. Differences between means were tested employing Duncan's multiple comparison test and significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Table – 1 shows effect of solvents on extraction process in connection with *in vitro* antibacterial activity of leaves of *Amaranthus spinosus* L. against *Staphylococcus aureus.* It was found out that methanol extract of the leaves of *Amaranthus spinosus* L. had maximum antibacterial activity against *Staphylococcus aureus.* With 30 µg/per disc and 60 µg per disc concentration of the methanol extract zone of inhibition came 30.6 ± 1.6 and 38.7 ± 1.5 diameter in mm respectively. Acetone extract followed by water had also good antibacterial activity against *Staphylococcus aureus.* Zone of inhibition with 30 µg/per disc and 60 µg per disc concentration of the acetone extract came 22.1 ± 1.3 and 28.8 ± 1.2 diameter in mm respectively and for water extract values for the same came as 21.3 ± 1.5 and 26.2 ± 1.3 diameter in mm respectively. Ethanol and petroleum ether extracts had, however, no significant effect on *in vitro* antibacterial activity against *Staphylococcus aureus.*

Effect of time on extraction process in connection with *in vitro* antibacterial activity of leaves of *Amaranthus spinosus* L. against *Staphylococcus aureus* is shown in Table – 2. It appears from the table that anti bacterial activity increased with time of extraction. This was up to one hour. Extraction for thirty minutes as well as for one hour gave zone of inhibition 20.3 ± 1.1 and 30.6 ± 1.4 respectively. There was, however, no significant change in anti bacterial property after one hour even when extraction time was extended up to two hours. Zones of inhibition after one and half hours as well as two hours period of extraction were 30.4 ± 1.7 and 30.5 ± 1.2 respectively.

Table – 3 shows effect of temperature on extraction process in connection with *in vitro* antibacterial activity of leaves of *Amaranthus spinosus* L. against

Staphylococcus aureus. Increase of temperature during extraction elevated anti bacterial activity. When extraction was done at thirty degree centigrade, zone of inhibition came 20.5 ± 1.6, at forty degree centigrade it was 30.8 ± 1.37. After that, increase in temperature during extraction had no effect on anti bacterial activity. Zone of inhibition came 30.9 ± 1.7 when temperature was increase to 50 ^{0}C.

Amaranthus spinosus L. a medicinal plant under the family of amaranthaceae, is distributed in lower to middle hills (3000–5000 ft) of entire north eastern Himalayas.The plant grows in cultivated areas as well as in waste places. Leaves of *A. spinosus* L. are stacked and alternate. The plant is known as "prickly amaranthus" in English and "ban lure"or "dhuti ghans" in Nepali. Medicinal uses of *A. spinosus* L. as mentioned in Ayurvedic text (Das AP and Ghosh Chandra, 2009; Chopra Col Sir RN & Chopra IC, 1958) are: Leaf infusion is diuretic and used in anemia. Root paste is used in gonorrhea, eczema, menorrhea etc. Besides, *A. spinosus* Linn. is used as laxative, diuretic, digestive and anti pyretic. It is also used to treat anorexia, leprosy, blood diseases, burning sensation, bronchitis, piles and leucorrhoea. The plant is further reported having anti-inflammatory properties, immunomodulatory activity and has effect on hematology (Kirtikar KR & Basu BD, 2001; Hussain Z et al., 2009; Olufemi BE et al., 2003; Tatiya AU et al., 2007; Assiak IE et al., 2002). Recent studies showed antidiabetic property of *A. spinosus* Linn. (Sangameswaran B & Jayakar B, 2008).

We also noted *In vitro* antibacterial activity of leaves of *Amaranthus spinosus* L. (Mitra Prasanta Kumar, 2014).

We, therefore, started isolation work of the antimicrobial compound present in *Amaranthus spinosus* L. responsible for anti microbial activity against *Staphylococcus aureus*. Extraction process is a part of isolation work. Different solvents yield different extracts and extract compositions (Girija K &Lakshman K, 2011; Zarnowski R and Suzuki Y, 2004). Therefore, a suitable extracting solvent should be selected for extraction of the active compound for its maximum activity (Wang L and Weller CL, 2006). We thus used distilled water, acetone, ethanol, methanol and petrolium ether separately as solvents for the extraction of the active compound from leaves of *Amaranthus spinosus* L. Results showed that methanol extract had maximum anti-microbial activity against *Staphylococcus aureus*. This was followed by acetone extract (Fig. 2).

As extraction time is important to extract active compound in maximum amount, we allowed extraction time as half an hour, one hour, one and half hours as well as two hours in separate experiment. It was found out that one hour extraction time produced the extract from leaves of *Amaranthus spinosus* L. which had maximum anti microbial activity against *Staphylococcus aureus*.

The extraction temperature is another important factor influencing the recovery of the bio reactive compound from the source (Cannell RJP, 1998; Huie CW, 2002). In separate experiments we thus conducted extraction at temperatures like thirty, forty and fifty degree centigrade. Results revealed that leaves of *Amaranthus spinosus* L. at 40^{0}C had more anti microbial activity against *Staphylococcus aureus* than the extraction temperature at 30 ^{0}C. Further increase of extraction-temperature, however, had no significant effect on the anti microbial activity against *Staphylococcus aureus*

We are now interested to see the effect of mixture of solvents on the process of extraction. Work is progressing in this direction.

Table 1. *In vitro* antibacterial activity of leaves of *Amaranthus spinosus* L against *Staphylococcus aureus* : Effect of solvents on extraction process.

Solvent	Dose (µg/disc)	Zone of inhibition (Diameter in mm)
Water	30	21.3 ± 1.5
	60	26.2 ± 1.3
Acetone (50%, v/v)	30	22.1 ± 1.3
	60	28.8 ± 1.2
Ethanol (50%, v/v)	30	18.1 ± 1.1
	60	19.4 ± 1.4
Methanol (50%, v/v)	30	30.6 ± 1.6
	60	38.7 ± 1.5
Petrolium ether (50%, v/v)	30	15.7 ± 0.9
	60	16.6 ± 1.0

Data shown in mean ± SEM (n = 5)

Table 2. *In vitro* antibacterial activity of leaves of *Amaranthus spinosus* L against *Staphylococcus aureus :* Effect of time on extraction process.

Solvent	Time (minutes)	Zone of inhibition (Diameter in mm)
Methanol (50%, v/v)	30	20.3 ± 1.1
	60	30.6 ± 1.4
	90	30.4 ± 1.7
	120	30..5 ± 1.2

Data shown in mean ± SEM (n = 5), Dose used - 30 µg/disc.

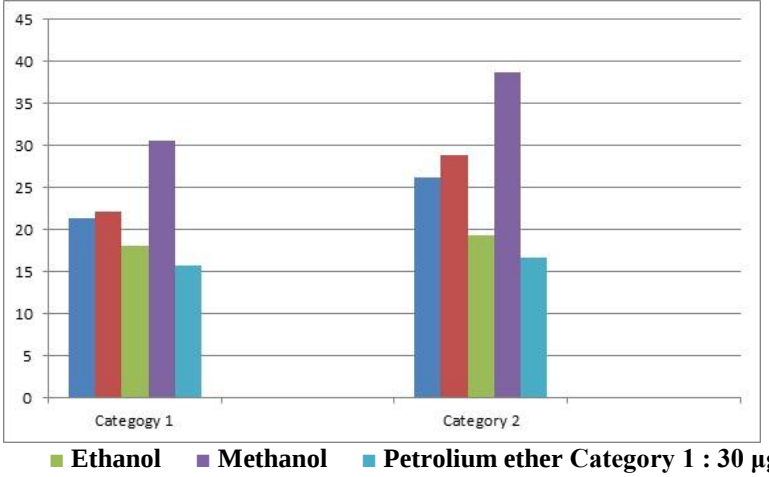
Table 3. *In vitro* antibacterial activity of leaves of *Amaranthus spinosus* L against *Staphylococcus aureus :* Effect of temperature on extraction process.

Solvent	Degree centigrade	Zone of inhibition (Diameter in mm)
Methanol (50%, v/v)	30	20.5 ± 1.6
	40	30.8 ± 1.37
	50	30.9 ± 1.7

Data shown in mean ± SEM (n = 5), Dose used - 30 µg/disc.

Fig. 1. *Amaranthus spinosus* L. Leaves

Figure 2. *In vitro* antibacterial activity (Zone of inhibition, Diameter in mm) of leaves of *Amaranthus spinosus* L against *Staphylococcus aureus :* Effect of solvents on extraction process.



Water Acetone Ethanol Methanol Petrolium ether Category 1 : 30 µg/disc Category 2 : 60 µg/disc

CONCLUSION

Extraction of leaves of *Amaranthus spinosus L.* with methanol (50%, v/v) at 40^0C for one hour had maximum antibacterial effect against *Staphylococcus aureus.*

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