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# DEVELOPMENT AND EVALUATION OF ANTIMICROBIAL FORMULATION CONTAINING EXTRACT OF *ANTHOCEPHALUS CADAMBA* (ROXB.) MIQ. LEAVES.

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**ABSTRACT**

The majority of the population in developing countries uses plants or plant preparations in their basic health care. Many plant species have been proved to have antimicrobial properties. However, several factors such as incorrect preparation, unstable preparation can interfere with the medicinal properties of plant and makes ineffective. The purpose of this study was to develop a stable preparation of well known Indian medicinal plant *Anthocephalus cadamba* (Roxb.) Miq. (*A. cadamba*) in the treatment of infectious skin diseases, by *in vitro* determination of their antimicrobial potential against selected microorganism. The results showed that the formulation containing 0.5% w/w showed good anti-microbial activity compared to other formulations. All formulations showed good stability during storage and no major changes observed after carrying out other physicochemical evaluations and during entire storage period.

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**Key Words**
*Anthocephalus cadamba;* antimicrobial activity; topical formulation.

**INTRODUCTION**

The use of medicinal plants in health care is as old as humanity. It is now known that primitive peoples constantly sought medicines from the plant kingdom to alleviate human suffering caused by disease or accidents. Common microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and some fungal strains are important etiologic agents for many diseases in the community (Ruhnke, 2006; Pires et al., 2007; Marcus et al., 2008; Nevet et al., 2010; Reddy et al., 2010). In present time, *s*ome strains of these microbes have become a leading cause of infectious diseases around the world, because of the development of strains resistant to many antibiotics; for example, methicillin-resistant *S. aureus* (MRSA) and carbapenem-resistant *P. aeruginosa* (Pichereau and Rose, 2010; Vitkauskienė et al., 2010). Thus, the search for plant products with high antimicrobial activity is of the utmost importance, as it provides an opportunity to find compounds with much greater activity than the drugs available today. *A. cadamba* (Roxb.) Miq. (Family-Rubiaceae) commonly called kadamba enjoys a hallowed position in Ayurveda- an Indian indigenous system of medicine. It primarily consists of indole alkaloids, terpenoids, sapogenins, saponins, terpenes, steroids, fats and reducing sugars. It was found that the almost all parts of the plant are used in the treatment of various diseases (nandkarni, 2006). Decoction of leaves is used as gargle in aphthae or stomatitis and in the treatment of ulcers, wounds, and having potent antimicrobial activity. Bark of the plant is used in fever, inflammation, cough, vomiting, diarrhoea, diabetes, burning sensation, diuresis, wounds, and ulcer and also in the treatment of snake-bite (ayurvedic pharmacopoeia 1999).

In present study attempts were made to develop topical formulation for antimicrobial action against various microorganisms like *S. aureus* which are associated with localized skin infection like boils, carbuncles and scalded skin syndrome, *E. coli* and *P. aeruginosa* which are associated with wound infections and burns (purohit, 2003 and tortora 2006).

**MATERIALS & METHODS**

***Collection and authentication of plant***

The plant was collected from central region of India (Bhopal) in the month of October. The plant was identified as authentic by Botanical survey of India, Allahabad with reference number: BSI/CRC/2010-11/245.

***Extraction***

The shade dried coarsely powdered leaves (400 g) first defatted with petroleum ether. The defatted leaves are now extracted with ethanol as a solvent using soxhlet apparatus. After extraction the solvent of total extract was distilled off and concentrate was evaporated on a water bath to a syrupy mass which then evaporated to dryness (Chandrasekhar and prasanna, 2009).

***Preparation of ointment***

The ointment was prepared by using the formulae as shown in table 1. Polyethylene glycol 400 and 4000 was selected as ointment base (Ugriné et al., 1989). Ointment was prepared by melting together polyethylene glycol 400 and 4000 on a hot plate/stirrer (at 70ºC). Drug was added to this molten base while stirring. The entire mixture was stirred while cooling to form ointment (Himal et al., 2010).

**Table 1**: Preparation of ointment using above mentioned formula.

| | | Quantity % W/W | | | |
|---|---|---|---|---|---|
| S. No. | Ingredients | F1 | F2 | F3 | F0 |
| 1 | PEG 400 | 70 | 70 | 70 | 70 |
| 2 | PEG 4000 | 30 | 30 | 30 | 30 |
| 3 | Leaf extract (*A.cadamba*) | 0.1 | 0.25 | 0.5 | -- |

F0 is control formulation.

***Evaluation of ointments***

***Spreadability determination***

Spreadability of formulation was determined by an apparatus which was fabricated in laboratory and used for the study. The ointment was placed between two glass slides and a weight of 1000g was placed on the slide for 5 minutes to compress the sample to a uniform thickness. Weight of 70g was added to weighing pan. Now the time in seconds required to move the slides was taken as the measure of Spreadability (Shrikande et al., 2007). The following formula is required for the calculation of Spreadability.

$S = m.l/t$

Where S = Spreadability, m = weight tied to upper slide, l = length of slide in cm and t = time taken to separate two slides.

***pH determination***

The pH of formulated ointment was determined using digital pH meter available in college (Panda et al., 2009).

***Viscosity determination***

100gm of each formulation was weighed and transferred to a beaker and viscosity was determined by using Brookfield dialed viscometer (USA), model RV, using spindle no. 7 at 50 RPM (Panda et al., 2009).

***Rheological study***

The rheology of selected formulation F3 was studied using the same viscometer. For ointment spindle no. 7 were found to be appropriate and different speeds of 0.5, 1.0, 2.0, 2.5, 4.0, 5.0, 10.0, 20.0, 50.0 and 100 were selected for rheological behaviour determination. Different torque values obtained at respective speeds for an ascending and descending graph. The rheogram obtained by plotting the rate of shear (per sec) on Y axis and shear rate (dyne/cm$^2$) on X axis. (Sinko, 2006).

***Stability study determination***

The formulations were subjected to accelerated stability testing study according to ICH guidelines for finished pharmaceutical products. The formulations were placed at 40$^o$ C ± 2$^o$ C at 75 % ± 5 % RH for a period of 3 months (Shakeel et al., 2008 and ICH Topic Q1 A (R2), 2003), and sample were evaluated for various parameters like pH, viscosity, Spreadability and organoleptic property at an interval of 15 days.

***Thermal cycling***

Most of the drug product, especially semisolid dosage form like ointment, creams, suspension and emulsions, lotions and suppositories may be adversely affected by variation in extreme temperature fluctuations. These types of drug products should be tested under cycling temperature condition to avoid this problem during storage and shipping. The prepared formulation was packed in container and placed in following storage conditions: 5$^o$ – 40$^o$ C in 24 h cycle for two weeks. The sample was tested for its organoleptic and physicochemical properties (Carstensen and Rhodes, 2008).

***Anti-microbial evaluation***

***Bacterial strain and inoculum preparation***

Bacterial cultures of *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphyllococcus aureus* (*S. aureus*) and fungal culture of *Aspergilus niger* (*A. niger*) were used for antimicrobial test organism. The bacteria were maintained on nutrient agar (NB) medium at 37$^o$C and fungus was maintained on potato dextrose agar (PDA) medium at 28$^o$C (Khalighi-Sigaroodi et al., 2005 and Fisgin et al., 2009).

***Antimicrobial activity assay***

The agar well diffusion method used for antimicrobial evaluation (Khan et al, 2007). The test microorganism was seeded into the respective medium by spread plate method. After solidification wells of 5 mm diameter were punched into medium and impregnated with the suppository sample. Streptomycin and tetracycline were used as positive control for bacteria and fluconazole for fungi. The plates were incubated at 37$^o$C for 24 h for bacteria and 28$^o$C for 72 h for fungi. Antimicrobial activity was evaluated by measuring the inhibition zone in mm (Umachigi et al., 2007). The entire test was performed in triplicate and the values corresponded to average ± S. D.

**RESULTS AND DISCUSSIONS**

The results obtained after physicochemical study are shown in table 2. The pH, viscosity and spreadability were found to be satisfactory and made it suitable for topical uses.

**Table 2**: Physicochemical evaluation of ointment.

| Formulation | pH | Viscosity (cps)* | Spreadability (g.cm/sec) | Rheology |
|---|---|---|---|---|
| F1 | 7.6 | 36000 | 10.08 | PSP |
| F2 | 7.45 | 37000 | 10.29 | PSP |
| F3 | 7.7 | 38000 | 10.8 | PSP |

* Spindle no. 7 @ 50 RPM, PSP = pseudoplastic flow

Figure 1 shows the rheological behaviour of formulation containing 0.55 w/w of drug extract. All the formulations showed pseudoplastic behaviour (viscosity is high under a low shear rate and low under a high shear rate).

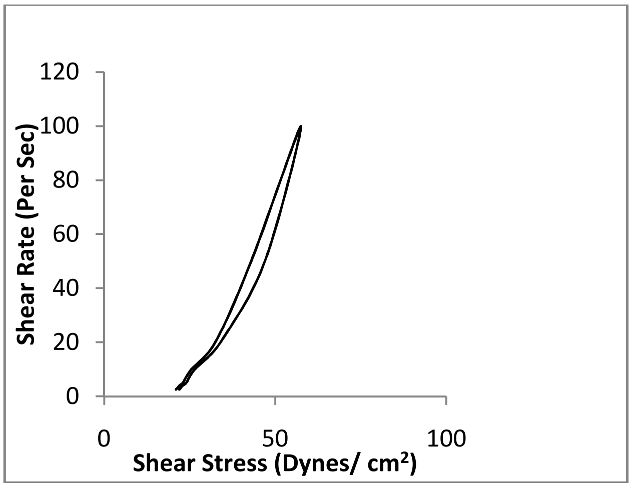



**Fig. 1**: Rheogram of formulation containing 0.5% w/w drug extract

All the formulations were stored at 40° C ± 2° C at 75 % ± 5 % RH for 90 days and evaluated regularly at an interval of 15 days were evaluated for different physicochemical parameters. No major changes was found during entire testing period and formulations remained stable when stored at 5° – 40° C in 24 h cycle for two weeks. The results are shown in table 3, 4, 5 and 6 respectively for F1, F2, F3 and thermal cycling.

**Table 3**: accelerated stability study of ointment containing 0.1% w/w of extract.

| Days/ Parameters | $F_1$ at 40° C ± 2° C at 75 % ± 5 % RH | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 45 | 60 | 75 | 90 |
| pH | 7.6 | 7.5 | 7.23 | 7.38 | 7.5 | 7.67 | 7.52 |
| Viscosity (cps)* | 36000 | 34500 | 32500 | 34000 | 33000 | 35500 | 35000 |
| Spreadability (g.cm/sec) | 10.08 | 10.23 | 10.19 | 10.35 | 10.43 | 10.27 | 10.28 |
| Colour | Brownish-Green | No change | No change | No change | No change | No change | No change |

*Spindle no 7 @ 50 rpm

**Table 4**: accelerated stability study of ointment containing 0.25% w/w of extract.

| Days/ Parameters | F2 at 40° C ± 2° C at 75 % ± 5 % RH | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 45 | 60 | 75 | 90 |
| pH | 7.45 | 7.30 | 7.22 | 7.59 | 7.41 | 7.44 | 7.14 |
| Viscosity (cps)* | 37000 | 35500 | 36000 | 36500 | 36000 | 34000 | 36500 |
| Spreadability (g.cm/sec) | 10.29 | 10.46 | 10.14 | 10.79 | 9.81 | 10.24 | 9.75 |
| Colour | Brownish-Green | No change | No change | No change | No change | No change | No change |

*Spindle no 7 @50 rpm

**Table 5**: accelerated stability study of ointment containing 0.5% w/w of extract.

| Days/ Parameters | $F_3$ at $40^{o}$ C ± $2^{o}$ C at 75 % ± 5 % RH | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 45 | 60 | 75 | 90 |
| pH | 7.7 | 7.95 | 7.26 | 7.37 | 7.81 | 7.52 | 7.65 |
| Viscosity (cps)* | 38000 | 37400 | 35000 | 36500 | 37000 | 35600 | 37500 |
| Spreadability (g.cm/sec) | 10.8 | 9.64 | 10.05 | 10.65 | 9.79 | 9.67 | 10.25 |
| Colour | Brownish-Green | No change | No change | No change | No change | No change | No change |

*Spindle no 7 @ 50 rpm

**Table 6**: Thermal cycling study of formulation.

| Parameters/ days | F1 | | | F2 | | | F3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 0 | 7 | 14 | 0 | 7 | 14 |
| pH | 7.6 | 7.4 | 7.5 | 7.45 | 7.6 | 7.35 | 7.7 | 7.65 | 7.8 |
| Viscosity (cps)* | 36000 | 35000 | 34500 | 37000 | 36400 | 35500 | 38000 | 36500 | 37000 |
| Spreadability | 10.08 | 10.21 | 10.20 | 10.29 | 10.26 | 10.35 | 10.80 | 10.31 | 9.54 |

* Spindle no 7 @ 50 rpm

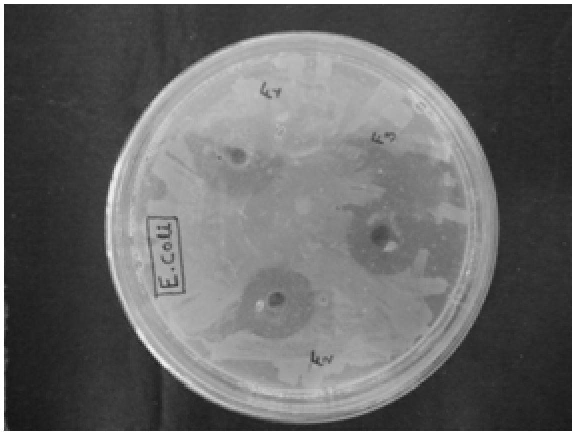
The anti-microbial activity of ointments was studied using different microbial species. It was observed that F3 has the maximum zone of inhibition against all the microbial species. The test formulations were also compared with available marketed preparation. The comparison showed that the formulation F3 is comparable with available synthetic marketed antimicrobial preparations. The results are as shown in table 7. The zones of inhibitions against selected microbes are shown in figure2.

**Table 7**: Anti-microbial activity of suppository for microorganisms with agar well diffusion method.

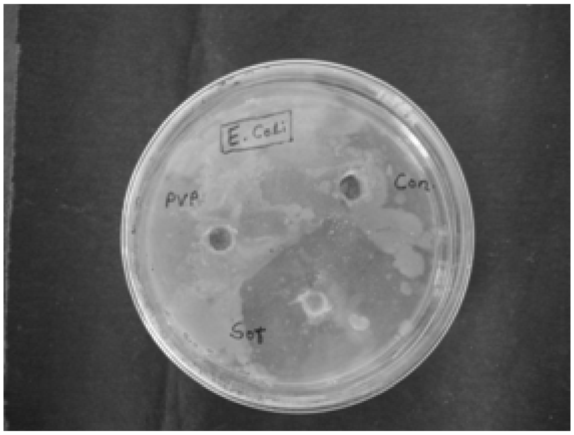
| Sr. No. | Microorganism | Zone of inhibition (mm)* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $F_1$ | $F_2$ | $F_3$ | S1 | S2 | S3 | C |
| 1 | *E. coli* | 11.16±0.15 | 12.23±0.05 | 14.13±0.15 | 16.1±0.1 | NA | NA | Growth |
| 2 | *P. aeruginosa* | 12.06±0.11 | 11.23±0.05 | 13.03±0.11 | 14.06±0.05 | NA | NA | Growth |
| 3 | *S. Aureus* | 11.10±0.1 | 12.03±0.05 | 12.06±0.11 | 14.13±0.05 | NA | NA | Growth |
| 4 | *A.Niger* | 11.10±0.1 | 11.06±0.05 | 10.06±0.05 | NA | NA | 12.06±0.05 | Growth |

*values reported as mean ± SD (n=3)

S1 = soframycin, S2 = betadine, S3 = miconazole cream, C = control, NA = not applicable




**A**

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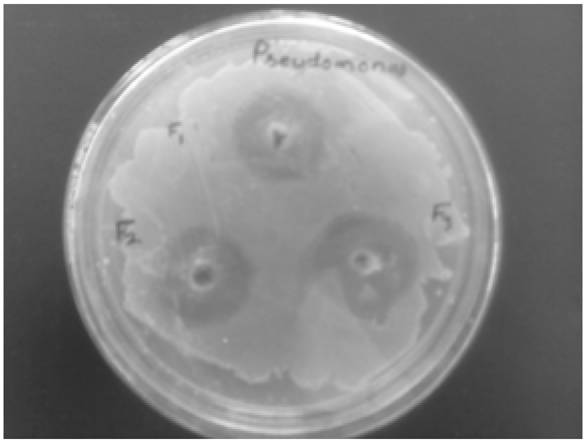
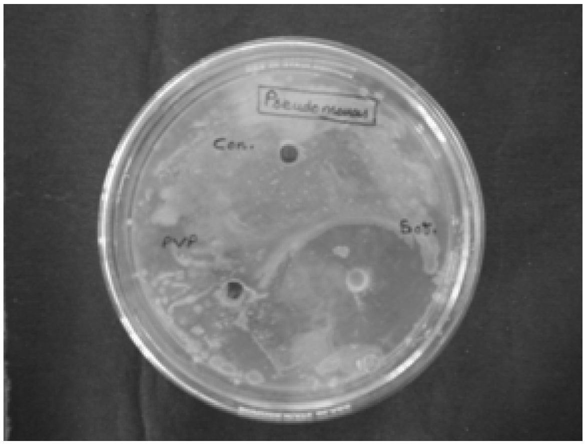



**C**

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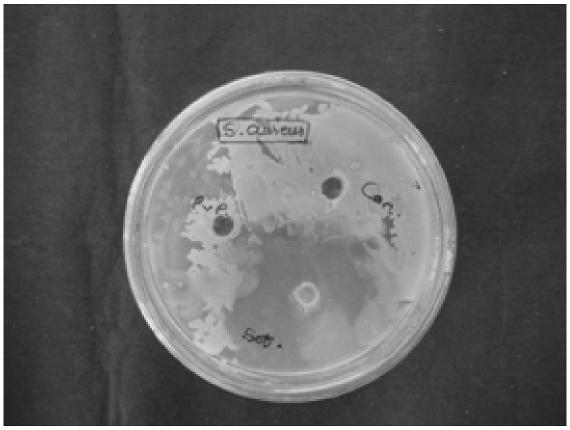
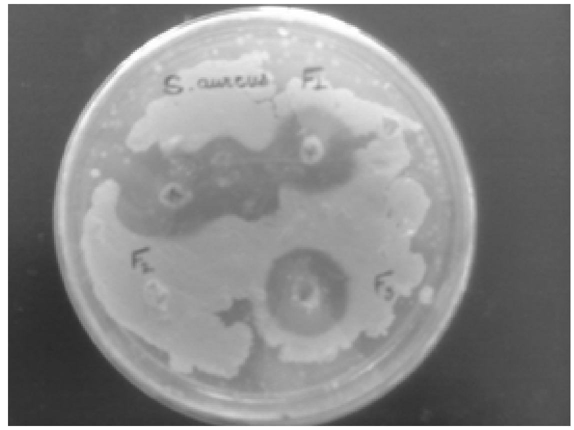
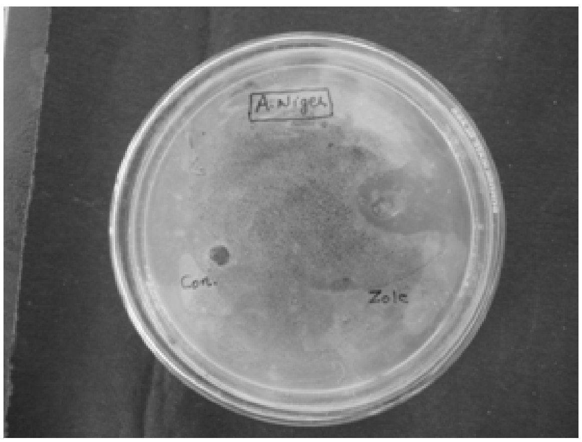


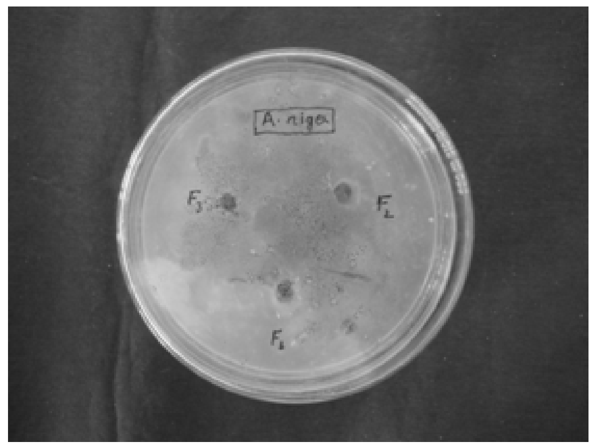

**E**




**F**




**G**



**H**

**Fig. 2:** Zone of inhibition against selected microbes. A= Zone of inhibition of formulations against *E.coli,* B= Zone of inhibition of standard and control drug, C= Zone of inhibition of formulations against *P. aeruginosa*, D= Zone of inhibition of standard and control drug, E= Zone of inhibition of standard and control drug, F= Zone of inhibition of formulations against *S. aureus,* G= Zone of inhibition of standard and control drug, Zone of inhibition of formulations against *A. niger*

## CONCLUSION

The results of present experimental study showed that it is possible to develop the antimicrobial ointment with alcoholic extract of *Anthocephalus cadamba* Roxb. Miq. leaves which is useful in the treatment of skin diseases caused by *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphyllococcus aureus* (*S. aureus*) and *Aspergilus niger* (*A. niger*).

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