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Anti-Inflammatory Effect of Cordia Myxa Extract on Bacteria that Infected Wounds in Rats as a Model for Human

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Abstract

The fruit extraction of Cordia myxa was done using ethanol. Ether of petroleum (40-60 percent), ethyl acetate, solvent ether, butanone and butanol in succession were used to further fractionate this extract. These fractions were tested for wound healing operation on sex of Wistar strain albino rats using the excision wound model. On the selected model, all the fractions reveled significant differences at ($P<0.001$).

Keywords: C. myxa, Flavonoids, Wound healing activity, Ethanol extract.

Introduction

An significant part of conventional medicine is the use of medicinal plants. One of these plants was formerly used in the 2nd and 3rd centuries A.D. in the Roman era. Cordia myxa is for its beneficial properties (Karami et al., 2015; Bouby et al., 2011). 'Bumber' is locally known, Cordia myxa fruit is one of largest genera in Boraginaceae family, as about three hundred species have been described globally, mostly in the worm region (Ali et al., 2015). It is found to develop primarily in Asia and around the world, especially at tropical areas with the right type of geophysical climate (Hamdia and Al-Faraji, 2017). The seeds of Cordia myxa are a good source of antioxidants present in daily life (Tian et al., 2014). Cordia myxa is a sweeter fruit since it contain the highest percentage of glucose, sucrose, fructose and high overall dietary fiber, which are play a major role in reducing the risk of several diseases (Aberoumand and Deokule, 2010). Cordia myxa fruit considered also as a rich protein source, fat, carbohydrates, ash, and important minerals like K, Na, Ca, Fe and also Zn (Karami, et al., 2015). The high levels of Cordia myxa are glycosides, sterols, flavonoids, saponins, alkaloids, trepenoids, phynolic acids, mucilage and gums (Jamkhande et al., 2013). So Cordia myxa fruit is widely used as diuretic, an anthelminthic, demulcent, astringent and expectorant agent for the treatment of chest, also urinary infections and wound healing (Kuppast and Vasudeva, 2006), as well as anti-inflammatory and essential biological activities and arthritic ant (Inas et al., 2011; Al-Musayeib et al., 2011). Therefore, this research focuses on the identification of the components of Cordia myxa and the assessment of microorganism inhibition function. Across the world, antibiotic resistance has become a serious public health problem. Nearly 2 million people in the USA develop nosocomial infections every year, resulting in 90,000 deaths. Over 70 percent of the bacteria that cause these diseases are resistant to at least one of the antibiotics commonly used throughout treatment (FDA, 2017). This makes the selection of the appropriate agent an increasingly challenging task that has made clinicians more dependent on in-vitro AST data (Shigemura et al., 2011). In the industrial world, the Kirby-Bauer disk diffusion method is a standard procedure for the susceptibility testing of bacterial

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isolates. When the test is performed following a standard procedure, it gives reliable results and can predict clinical efficacy of the antibiotics tested (King and Brown, 2001).

Materials and methods

Fruit (10kg) with C. Myxa shade was dried and a coarse powder prepared. In batches of ethanol, the fruit powder was exposed to repeated soxhlation. The alcoholic extract was suspended after complete extraction and further fractionated successively using petroleum ether (40-60 percent), solvent ether, ethyl acetate, butanol, and butanone. For petroleum ether, solvent ether, ethyl acetate, butanol and butanone, the percentage yield for fractions was 15, 8, 12, 7.5 and 5.2g, respectively. Such fractions were vacuum dried and used for the excision wound model to study wound healing properties. Tween-80 (1%) was used as a vehicle for suspension of the fraction concentrates for incision wound. The positive test for flavonoids was found in ethanol extract and ethyl acetate and butanol fractions of fruits. Shinodha and Zn-HCl reduction experiments have examined the existence of flavonoids and reported TLC studies using Silica Gel-G as a stationary step and a mixture of chloroform: acetone: formic acid (70:20:10) as a mobile phase. After activation, anisaldehyde sulphuric acid was sprayed to label the flavonoid spots. The flavonoids were isolated by column chromatography technology for further analysis. After that, the isolated pure compounds for structural elucidation were subjected to spectral analysis. Activation by spraying anisaldehyde sulphuric acid was found in the flavonoid spots. The flavonoids were isolated by column chromatography technology for further analysis. Finally, the isolated pure compounds for structural elucidation were subjected to spectral analysis. 35 Albino rats of either sex weighing 140-190 g were divided into 7 groups used to determine the dose. Rats were fasted overnight prior to the toxicity trial where the dose determination process of "Up and Down" was used. Tween-80 (1 percent) was used to suspend the fractions and was given orally as a vehicle. Food was made available in the form of dry pellets and water ad libitum. Basal food intake and body weight were reported to the nearest gram. Wounding was conducted aseptically under light ether anesthesia. The method of excision was used to evaluate wound healing activity (Ghosh, 1984). Prior to excision, the rats were anesthetized and eventually sacrificed by exposure to a higher dose of anesthetic ether prior to the assessment of the tensile strengths of the resutured wounds and the removal of granuloma tissues (grass pith). Each group comprising 5 animals were used in the excision wound analysis (Morton and Malone, 1972). The circular wound has a diameter of around 3 cm. It was rendered in aseptic condition on the dorsal aspect area of rats under light ether anesthesia and observed during the study. The animals were individually housed. Suspensions of Tween-80 (1%) extracts prepared under analysis were administered daily for 15 days starting on the day of the wound. The microorganisms were cultured from the wound that done on the rats, the media that used were Nutrient broth, nutrient agar, Sabouraud dextrose agar and malt extract broth from Himedia Laboratories, Mumbai (India). The antibiotic discs that used in this study were gentamycin, chloramphenicol, trimethoprim, sulfa, penicillin and vancomycin.

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Statistical Analysis

The Application Statistical Analysis Pattern SAS (2012) has been used to detect the effect of differentiating factors on the study parameters. The least significant difference – the LSD test (Analysis of Variation-ANOVA) was used to make a significant comparison between the means.

Results and discussion

The results were analyzed by "t" test at a significance level of $P<0.001$. The result of excision wound on 5^{th}, 9^{th}, 11^{th}, 13^{th} and 15^{th} days are summarized in Table 1. Contractility of excision wound was promoted from 5th day after treatment to 15th day after treatment. In the case of treated rats with fractions of fruit ethanol extract, the epithelization of the wound was noticed to be much earlier than control. A massive increase in the tensile strength of the test compared to the control shows that the extracts promote wound healing activity. The results of the excision wound model exhibited substantial wound healing properties of the Cordia myxa fruit extracts. Our results for Cordia myxa ethanol extracts and fractions were agreed with Kuppast and Nayak (2006), whom calculate the percentage closure at 4^{th}, 8^{th}, 10^{th}, 12^{th} and 14^{th} day post wounding and by using C. dichotoma extract. Recent studies on wound healing action claim that flavonoids facilitate major wound healing properties (De Groot, 1994). Increased production of reactive oxygen throughout injury resulting in the intake and depletion of endogenous scavenging compounds and flavonoids could have an additive effect on endogenous scavenging compounds. Flavonoids may also boost the role of endogenous antioxidants, It is also found that the flavonoid hydroxyl groups make radical inactive by combining flavonoids [OH] + R with radical flavonoids [O] + RH. From this study we concluded that wound healing activity of the fruit extract of Cordia myxa may be due to the presence of flavonoids (Korkina and Afanas, 1996). The result of our study according to in vitro zone of inhibition when using of C. myxa and different types of antibiotic discs were summarized in table 2. The zone of inhibition of C. myxa extract (diameter 34 mm) gave higher significant differences ($P<0.05$) as compared to other antibiotic discs, this results were disagreed with Nariya et al. (2011), whom found that diameter was 20 mm, this may be due to the concentration of extract or the type of Cordia species. Our result were agreed with Jasiem et al. (2016).

Table 1: Mean percentage closure of excised wound area in mm^2 ± S.E. following post wounding days by Cordia myxa ethanol extracts and fractions

Extract and Fraction	Day 5th	Day 9th	Day 11th	Day 13th	Day 15th
Control group	24.87±1.11	49.63±0.56	64.45±1.45	79.53±1.71	88.96±0.78
Ethanol	38.35±2.01	65.56±1.22	79.99±0.33	87.39±0.46	91.39±0.16
Pet. ether	40.93±2.30	75.40±0.99	86.64±0.59	96.69±0.21	98.42±0.37
Solvent ether	63.11±1.11	73.33±2.22	81.53±1.32	99.16±1.07	99.92±0.10
Ethyl acetate	48.37±0.98	61.32±1.19	84.99±1.84	93.01±1.43	97.23±0.39
Butanol	64.21±0.59	79.98±2.02	89.34±0.19	98.01±1.31	98.99±0.08
Butanone	47.91±2.17	55.76±1.27	79.72±0.48	86.75±0.61	93.26±1.63

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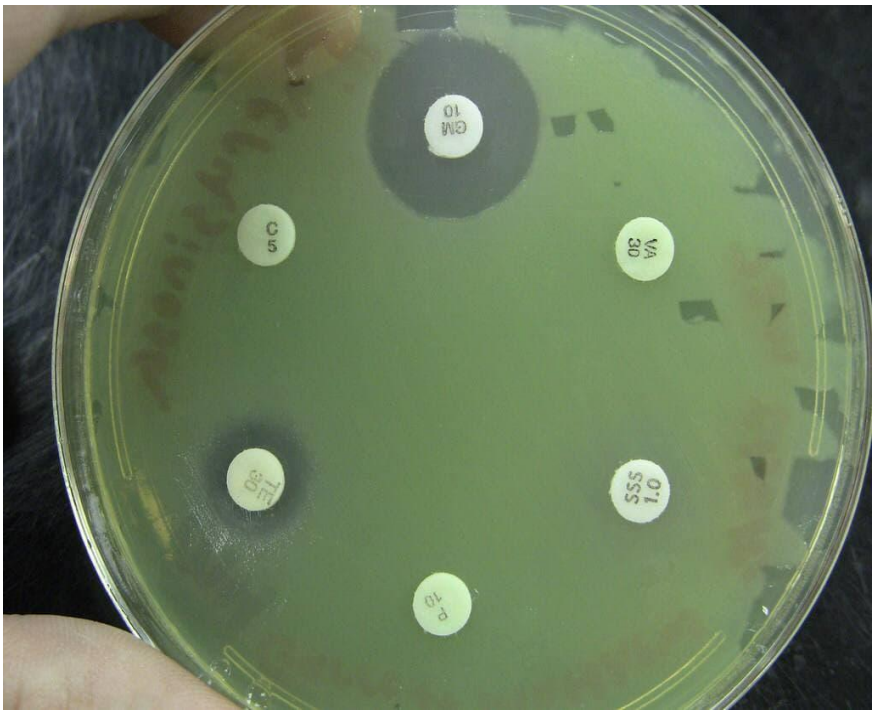
Table 2: Zone of inhibition that occur after using of C. myxa extract, gentamycin, chloramphenicol, trimethoprim, sulfa, penicillin and vancomycin on Pseudomonas aeruginosa in vitro.

Discs	Zone of inhibition (diameter/ mm)	Sensitivity	Resistant
C. myxa extract	34 a	100%	0%
gentamycin	21 b	70%	30%
chloramphenicol	0	0	0
trimethoprim	10 c	20%	80%
sulfa	0	0	0
penicillin	0	0	0
vancomycin	0	0	0

($P<0.05$)



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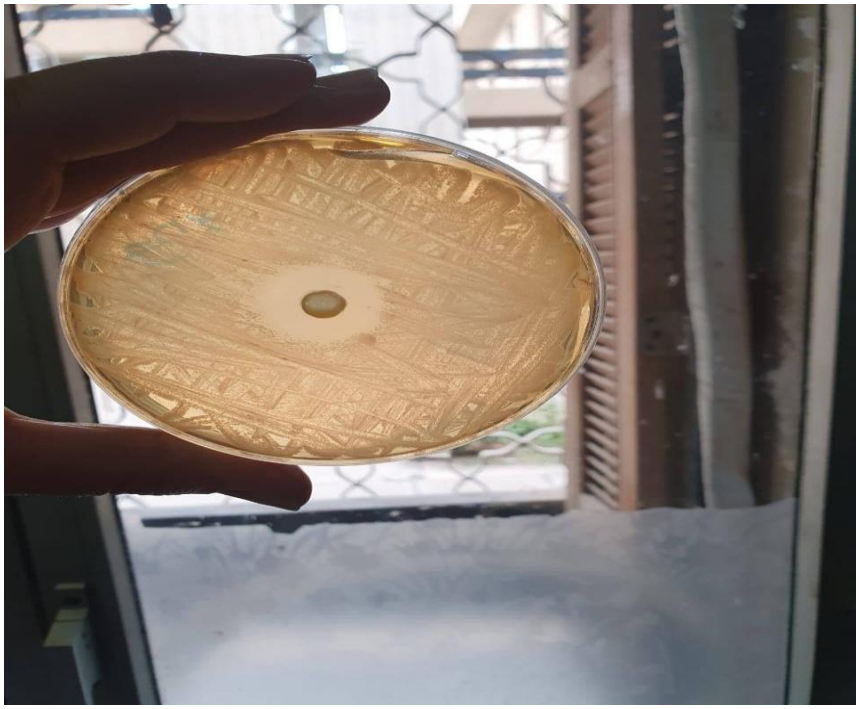

Figure 1: Inhibition zone diameter for C. myxa extract and different types of antibiotics.

Conclusion

From this study we concluded that the using of Cordia myxa ethanol extracts and fractions would be accelerate wound healing and gave a result better than different types of antibiotics.

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