Comparative antibacterial activity of *Morus alba* L leaves and fruits extract.

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Abstract

Antimicrobial proficiency of *Morus alba* L. a therapeutic plant (leaf and fruit extract) were examined utilizing ethanol, as solvents and tested against 4 human pathogens bacteria: *Staphylococcus aureus, Streptococcus mutans, Pseudomonas plecoglossida, Pseudomonas aeruginosa* utilizing agar well diffusion strategy. The plants demonstrated huge action against all pathogens, however the ethanolic concentrate of *M. alba* indicated most extreme zone of inhibition against each microorganisms. The maxium zone of inhibition was found in ethanolic leaves extract where as less antimicrobial action was found against ethanolic fruits extracts. The Spectrum of antibacterial activity has shown that the present examination might be demonstrative of the present investigation, ethanolic concentrates of these plants could be a conceivable source to acquire new and successful home grown drugs to treat diseases, consequently advocated the ethnic importance of *Morus alba* against different irresistible ailments.

Keywords: Antibacterial activity, *Morus alba* L. therapeutic plant, Agar well diffusion method .

Introduction

With the advancement in Science and Technology, noteworthy advancement has been made in the field of prescription with the revelations of numerous characteristic and manufactured drugs. Antibiotics are irrefutably a standout amongst the most critical restorative discoveries of the twentieth century that had effectiveness against serious bacterial diseases. Nonetheless, just a one third of the infections known have been treated from these synthetic products. This is a direct result of the rise of resistant pathogens,that is certain the outcome of long boundless unpredictable utilize, ceaseless and abuse of antibiotics. Antibiotic resistance has expanded considerably in the ongoing years and is representing a consistently expanding restorative issue. One of the strategies to lessen resistance to antibiotics is by utilizing antibiotic resistance inhibitors from plants. Plants are known to create an assortment of mixes to secure themselves against an assortment of pathogens. Medicinal plants have been utilized as customary medications for various human diseases for many years and in numerous parts of the world. Consequently, analysts have recently focused on more secure phytomedicines and organically dynamic mixes detached from plant species utilized. Mulberry is the one of the most studied plants for pharmacological potential. *Morus alba* shows the presence of wide range of phytochemicals responsible for the antioxidative potential of *Morus alba*. *Morus*

alba fruits are considered as nutritious food with many flavonoids and polyphenols and the important ones identified are apigenin, quercetin, luteolin, morin, umbelliferone, caffeic acid, rutin, gallic acid, chlorogenic acid and kaempferol Arfan *et al*.,(2012), Chu *et al.*, (2006) associated with cardiovascular disease for over a millennium in eastern countries.Extracts of this plant were used in the herbal prescriptionsby Chinese people to reduce blood pressure. The leaf extract shown to reduce hypertension in rodents and decreases serum cholesterol and prevent atherosclerosis. Prenylated flavonoids isolated from *M. alba* showed antibacterial, antiviral and antifungal activities. Kuwanon G was isolated from the ethyl acetate fraction of methanol extract of *M. alba* is acting as an antibacterial agent against oral pathogens. *Morus alba* has a long history that started with folk prescription and during that time has been consolidated into conventional and allopathic medicine1. Since numerous plant species are reported to have pharmacological properties as they are known to forces different secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, tirpenes which is thusly, ought to be used to fight the disease causing pathogens. Du *et al*., (2003) Sohn *et al*.,(2004) Sohn *et al* .,(2004).

MATERIAL AND METHODS

Collection of plant material

Mature plants of *Morus alba* utilized for this examination was collected from its natural habitat (Hoshangabad,M.P). Extraction of *Morus alba* leaves and fruits were done using soxhlet method (leaves) and maceration (fruits).

Culture and Maintenance of microorganisms

Unadulterated cultures of every single test bacteria were acquired from Scane Research Labotatories Bhopal,M.P. India. The unadulterated bacterial cultures were kept up on nutrient agar medium. Each bacterial culture was additionally maintained consistently on a similar medium and stored at 4^{o} C before utilize in study.

Preperation of plant extract

Leaves of *Morus alba* gathered from source plant were washed with running tape water and lastly with refined water for 2 times, trailed by ethanol wash and afterward permitted to dry at 50°C for overnight lastly processed to a coarse powder. 100 gm of powdered material (leaves) was soxhlet separated and macerated (fruits) for 24 hours. Each concentrate were evaporated in vacuum under low pressure.All concentrates were put in a clean glass bottles at room temperature until screened.

Microbiological screening

Antimicrobial actions of various concentrates were assessed by the agar well diffusion method by Olurinola (1996) with little modification.

Media Preapration and Its Sterilization

For agar well diffusion strategy by Olurinola, (1996) antimicrobial vulnerability was tried on solid (Agar-agar) media in petri plates. For bacterial test nutrient agar (NA) (40 gm/L) was utilized for creating surface colony development. The suspension culture, for bacterial cells development was done by preparing 2% Lauria Broth (w/v). The media prepared was then sterilized via autoclaving the media at (120°C) for 20 min.

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Agar well diffusion technique

Agar well-diffusion technique was pursued to determine the antimicrobial action. Nutrient agar (NA) plates were swabbed with sterile cotton swabs, with 9 hour old broth culture of individual bacteria. Wells of 10mm diameter were made in every one of these plates utilizing sterile stopper borer. Stock solution of ethanolic plant separates was set up at a concentration of 1 mg/ml. Around 100 µl of concentrations of plant solvent extracts were included using clean syringe into the wells and permitted to diffuse at room temperature for 2hrs. Control tests containing inoculums without plant extract were set up. The plates were brooded at 37°C for 24 h. The width of the inhibition zone (mm) was estimated and the action index was likewise computed. Triplicates were kept up and the examination was rehashed thrice, for each replicates the readings were taken in three distinctive settled directions and the average values were recorded.

Test for antibacterial activity.

The antibacterial activity was completed by microdilution technique with the end goal to decide the antibacterial action of mixes tried against the pathogenic bacteria. The bacterial suspensions were balanced with sterile saline to a concentration of 1.0 X 107 CFU/ml. The inocula were arranged and stored at 4 oC until utilize. Dilutions of the inocula were cultured on solid medium to confirm the nonattendance of contamination and to check the legitimacy of the inoculum. All tests were performed 2 times and rehashed three times.

Result and Discussion.

Estimation of antimicrobial action utilizing Agar well diffusion method

In the present investigation, the inhibitoy effect of both ethanolic leaves and fruits extract of *Morus alba* were evaluated against two gram positive (*Staphylococcus aureus ,Streptococcus mutans)* and two gram negative bacterial strains *(Pseudomonas plecoglossida, Pseudomonas aeruginosa).*The antimicrobial action

was determined using agar well diffusion method Table 1.1.The antimicrobial capability of both leaves and fruits was assessed by their zone of inhibition against different pathogens and the outcomes i.e. zone of inhibition were contrasted with the activity of the standards, viz., Ciprofloxacin and Ofloxacin. The outcomes uncovered that each extract are intense antimicrobials against each microorganisms examined. In ethanolic leaves extract of *Morus alba* the zone of inhibition was found in following order: *Streptococcus mutans* (31.33±1.15) > *Staphylococcus aureus* (27±1.00) >*Pseudomonas plecoglossida* (*14*.67±1.53)> *Pseudomonas aeruginosa* (17±1.00) .Where as in fruits the zone of inhibition was found in following order: *Streptococcus mutans* (31.32±1.15)>*Pseudomonas plecoglossida* (12.33±0.58) >*Staphylococcus aureus* (8.33±0.58) >*Pseudomonas aeruginosa*(8.17±0.76).In comparision to the leaves and fruits extract of *Morus alba*,the maxium zone of inhibition was found in ethanolic leaves extract against *Streptococcus mutans* (31.33±1.15).Current findings are similar to the results documented by Khalid *et al* (2011) in *Morus nigra*.

Table 1.1: Antimicrobial activity of ethanolic extract of *morus alba* L. on selected microbes

S. No.	**Name of microbes**	**Zone of inhibition**		
		25mg/ml	**50 mg/ml**	**100mg/ml**
	Ethanolic extract (Leaves)			
1.	*Pseudomonas plecoglossida*	25.33±0.58	21.33±0.58	14.67±1.53
2.	*Pseudomonas aeruginosa*	6.27±0.46	9.67±1.53	17±1.00
3.	*Streptococcus mutans*	7.33±0.58	29.00±1.00	31.33±1.15
4.	*Staphylococcus aureus*	7.33±0.58	18.33±0.58	27±1.00
	Ethanolic extract (Fruits)			
1.	*Pseudomonas plecoglossida*	8.67±1.15	10.67±1.15	12.33±0.58
2.	Pseudomonas aeruginosa	6.17±0.29	6.67±0.58	8.17±0.76
3.	*Streptococcus mutans*	27.33±0.58	29.00±1.00	31.32±1.15
4.	*Staphylococcus aureus*	7.33±0.58	11.33±1.151	8.33±0.58

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