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Phytochemical screening and antibacterial study of stem bark of *Morus australis* Poir.

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Abstract

The basic aim of the present study was to screen for phytochemical constituents (phenol and flavonoid content) and antibacterial activity of methanolicextract of*M. australis*poir.stem barkPhytochemical screening included the qualitative test by colour reactions and quantitative estimation of total polyphenolic and flavonoid content of methanolicextract of plant.The result of the study revealed that methanolic extract of *M. australis*poir. hadtotal phenolic and flavonoid content (266.79± 0.01 mg of GAE/ g and 15.55± 0.02 mg of QAE/g respectively).Biological screening include antibacterial activity testing by well diffusion method.The effects of methanolic extracts on some pathogenic bacterial strains viz*: staphylococcus aureus,Escherichia coli, Methicillin resistant staphylococcus aureus, pseudomonas aeruginosa* MDR, *klebsellapnemoniae, Klebsella pneumonia*MDR showed strong activity thus can be used to treat infections caused by these bacteria. The effectiveness of the crudemethanolic extract confirmed its use in traditional medicine to treat skin, urinary tract, diarrheal infections.Thus, needsfurther exploration for their effective use in both modern and traditional system of medicines.

Keywords: TPC and TFC content, Antibacterial, *Morus australis* Poir.

Introduction

Herbal medicine, the oldest form of healthcarestill play vital role in the treatment of many infectious diseases [Lambart et.al 1997]. The treatment with the herbal medicine are closely linked with nature.According to WHO, around 80% of population relies on herbal medicine, which is the biggest advantage, as an alternative source of medicine [Luitel et.al 2014].Due to continuous use of antibiotics, microorganism has become resistant and this creates a world-wide problem. To solve this problem, there is need to screen the local medicinal plants to develop alternative antimicrobial drugs for the treatment of infectious diseases.

Morus is a small genus consisting of about 13 species which belong to the family Moraceae. This genus contains a variety of biochemical compounds such as flavoinds, phenolic compounds that exhibits interesting biological activities like antimicrobial, anti-inflammatory, diuretics etc. [Nomura et. al 1988].

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Material and Methods

Plant collection

The barks of the *M. australis* was collected from Kathmandu district in the month of August (2017) which was duly identified as *Morusaustralis*poir.in National Herbarium and Plant Laboratory, Godavari, Lalitpur, Nepal and authenticated (Voucher specimen No. 212).

Preparation of plant extract

The barks of *M. australis* was cut into pieces and dried in room temperature. Dried sample was then crushed into powder by grinder. The powder was passed throughsieve No. 30 and stored in airtight container for further use.

Extraction was done by cold maceration process and dried by evaporation under reduced pressure.

Phytochemical screening

The phytochemical screening included qualitative tests by color reactions and Quantitative estimation of total phenolic and flavonoid content of plant extract.

Qualitative phytochemical screening

The qualitative phytochemical was done to identify the main groups of chemical constituents present in different extracts by their color reactions with different reagents. Each extract was subjected for glycosides, alkaloids, saponin, carbohydrate, tannin, protein, phytosterol, reducing sugar, flavonoid, phenol test to check the absence or presence of

secondary metabolites. These tests were done using a standard procedure [Roopashree et.al 2008,Joubert et. al, 2008]

Quantitative determination of TPC and TFC

Determination of TFC

Total Flavonoid Content of the plant extracts was determined by according to the colorimetric method [Adedapo, A et. al.]. Briefly 20µl of extracts (5mg/ml) was separately mixed with 5µl ethanol, 5µl aluminum trichloride (AlCl3, 10%). Subsequently 5µl of 1 M potassium acetate and 110µl distilled water was added into each well and the reactions mixture was allowed to stand for 30 minutes. Then the absorbance was measured in 415 nm with UV-Visible spectrophotometer. quercetin was used for constructing the standard curve (10-50µg/ml) and the total flavonoid compounds concentration in the extracts was expressed as miligrams of quercetin equivalent per gram of dry weight (mg QE/g) of extract. For extracts triplicate was performed to check and get more accurate results

Determination of TPC

The total phenolic content of the extract was measured using Folin-Ciocalteu reagent (Swamy MK et al.). Briefly, 20µL of the plant extract (0.5mg/ml) was separately mixed with 60µL of Folin-Ciocalteu reagent and 80 µL aqueous sodium carbonate (1M) solutions. Then the mixture was allowed to stand for 15 minutes at room temperature. The absorbance of the reaction mixture was measured at 765nm using Spectrophotometer, gallic acid was used for constructing the standard curve (10-80µg/ml) and the total polyphenolic compound concentration in the extract was expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g) of the extract using gallic acid standard curve. For the extract, triplicate was performed to check the reproducibility of the experimental result to get more accurate result.

Antibacterial activity

Antibacterial assay of extract was performed by agar well diffusion method in Muller Hinton Agar (MHA).

In this study, screening and evaluation of anti-bacterial activity was performed by agar well diffusion method. At first, MHA plates of approximately 4mm thickness was prepared. Sterile cotton swab was dipped into the prepared inoculums and carpet culture was done. Wells were made in the inoculated media plates and labeled properly. The diameter of well was 6mm. 50µl of the working solution (25mg/ml, 12.5 mg/ml, 6.25mg/ml, and 3.125mg/ml) of the plant extracts were loaded into the respective wells with the help of micropipette. DMSO was used as negative control and Neomycin was used as positive control. The plates were incubated at 37 degree Celsius for 12-18 hours. After overnight incubation, the zone of inhibition were observed and the diameter of ≥12 mm was considered as having antibacterial activity [Kusuma et al., 2017].Each assay was performed using triplicate and mean, all the three experiments were taken.

Results and Discussion

Phytochemical extraction

The percentage yield of the plant extract of *M. australis* stem bark was found to be 6.70%.

Phytochemical screening of plant extract

Qualitative phytochemical screening of the plant was carried out using color forming and precipitating chemical reagents for detecting plant constituents from their extracts .The result obtained were tabulated as follows, indicating the presence of different group of active constituents in methanol extracts.

Table 1: Phytochemical screening of plant extract

Phytoconstituents	Methanolic extract
Alkaloids	_
Flavonoids	+
Tannins	+
Saponins	+
Terpenoids	+
Phenol test	+
Glycosides	+
Proteins	+
Carbohydrates	+

+ = Presence: - = Absence

Fig. 1: ***Phytochemical test of stem bark of M. australis*** **Poir.**

Determination of total flavonoid content

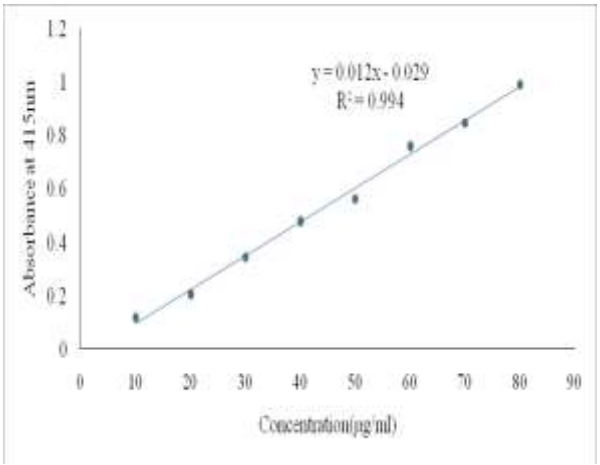

Fig. 2: Calibration curve of quercetin

Determination of total phenolic content

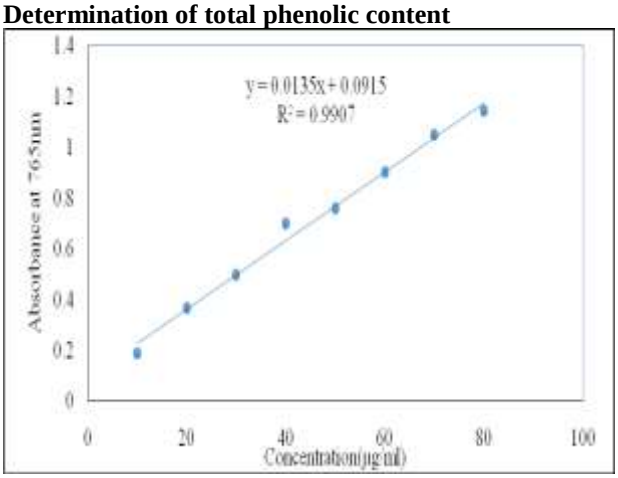

Fig. 3: Calibration curve of gallic acid

Table 2: Result of TFC and TPC

Extract	TPC (mg GAE/ g of wt of extract)	TFC (mg QE / g of wt of extract)
Methanolic extract	266.79± 0.01	15.55±0.02

Antibacterial activity

Antibacterial activity were tested against gram positive organism; *Staphylococcus aureus* (ATCC 25923), gram negative organism; *Escherichia coli (ATCC 25922), Klebsellapneumoniae (ATCC 700603)* and multi-resistant bacteria; *Methicillin Resistant Staphylococcus aureus (*MRSA*), Klebsellapneumoniae MDR, Pseudomonas aeruginosa*MDRusing Neomycin as standard.

The following table shows the result of preliminary antibacterial activity of plant extract.

Table 3: Antibacterial activity of plant extract

Microorganism	Methanolic Extract (mg/ml) Zone of inhibition, mm (Mean± S.E)				Control*
	3.125 (0.15mg /well)	6.25 (0.3mg/ well)	12.5 (0.62mg/ well)	25 (1.25mg /well)	20µg/ml (0.001 mg/ well)
S. aureus	15.1±0.02	16.3±0.18	17.5±0.2	19.5 ±0.1	19.3±0.10
E.coli	14.5±0.1	16.2±0.18	17.3±0.1	19.3 ±0.2	19.5±0.15
K. pneumoniae	17.6±0.18	18.6±0.15	20.5±0.05	22.6 ±0.10	17.1±0.10
MRSA	16.2±0.18	17.3±0.3	18.5±0.1	21.1 ±0.1	14.5±0.18
*P.aeruginosa (*MDR*)*	17.7± 0.18	18.5±0.10	20.3±0.10	22.3 ±0.1	11.2±0.10
*K.pneumoniae (*MDR*)*	17.6±0.15	19.1±0.01	20±0.02	21.4 ±0.1	16.5±0.2

*Control = Neomycin, Diameter of well = 6mm

The present study dealt with phytochemical analysis and antibacterial activity of *M. australis*poir. stem bark. The constituents of stem bark of *M. australis*was extracted with methanol by cold maceration process having the percentage yield 6.70%. Phytochemical screening tests showed presence of different kinds of phytochemical constituents that could act as a lead compounds for the discovery of different classes of possibly potent and effective therapeutic agents which can used against various diseases. Thus, Phytochemicals constituents play most important role in medicinal properties of plants. Phenolic compounds are responsible for wide range of pharmaceutical properties such as antioxidant, anticancer, anti-inflammatory, antimicrobials. Saponins containing steroids or triterpenoids reported to have excellent biological activities such as anti-diabetic, anti-inflammatory, antimalarial, and anticancer. [Ines Thabti et al.].The test results revealed that methanolic extract of *M. australis*stem bark have flavonoid, tannin, phenol, saponins, glycosides, protein, carbohydrate but absence of alkaloids which showed the presence of most of these bioactive compounds attributed to have potential antibacterial, and antioxidant properties.

The total phenolic content (TPC) and total flavonoid content (TFC) of the extracts were expressed as

milligram of Gallic Acid Equivalent per gram of extract i.e mg of GAE/g extract and milligram of Quercetin Equivalent per gram of extract i.e mg QAE/g extract respectively. The amount of total phenolics and flavonoids of *M. alba*, measured by Folin-Ciocalteau and Colorimetric assay, which was found to be 266.79± 0.01 mg of GAE/ g and 15.55± 0.02 mg of QAE/g respectively.

The antibacterial activity of plant extract was studied quantitatively by measuring the diameter of zone of inhibition.The antibacterial potential of the plant extracts were studied with four different concentrations (3.125mg/ml, 6.25mg/ml, 12.5mg/ml, 25mg/ml).

Fig. 4: Antibacterial activity of plant extract against *S. aureus*

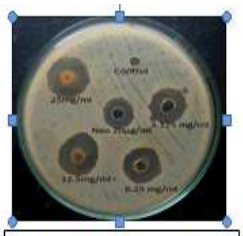

Fig. 5: Antibacterial activity of plant extract against MRSA

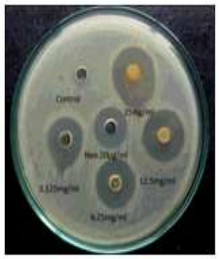

Fig. 6: Antibacterial activity of plant extract against *K.Pneumoniae*

Fig. 7: Antibacterial activity of plant extract against *K.Pneumoniae* MDR

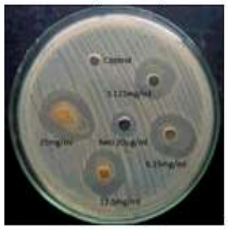

Fig. 8: Antibacterial activity of plant extract against *P. aeruginosa* MDR

The antibacterial screening of methanolic extracts of *M.australis*showed potent activity against both tested gram positive (*Staphylococcus aureus*) and gram negative bacteria *(Escherichia coli, Klebsellapneumoniae*). In addition, it also showed potent activity against the multi-drug resistance i.e*P.aeruginosa*MDR, MRSA, *K.pneumoniae MDR*.From the results, it was found that plant extract showed strong activity at increasing concentration against almost all tested organism. The methanolic extract against *K. pneumonia* and *P. aeruginosa* MDR, *K. pneumonia* MDRshows excellent activity as compared to MRSA, *S. aureus*, *E.coli* ranging from 17- 23 mm. This shows that plant extracts was more susceptible against gram negative organisms when compared to gram positive organisms especially against multi-drug resistant.

Conclusion

From this study, it was found that the plant possessed different phytochemical constituents like saponins, glycosides, terpenoids, flavonoids, tannins, carbohydrates and phenol which are responsible for biological activities.From this study, it wasalso found that the plant extract showed potent antimicrobial activity against tested organisms in dose dependent manner which supports the traditional use of this plant as broad spectrum of activity.

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