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Antibacterial Screening of *Tinospora cordifolia, Withania somnifera, Centella asiatica, Azadirachta indica* And Their Poly Herbal Formulations

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ABSTRACT

Herbal drugs are tremendously explored for anti-infective compounds as the emergence of multidrug resistant bacteria are prevalent. The present research was carried out to validate these claims and to develop a polyherbal formulation. Concentrated alcoholic and aqueous extract of the polyherb containing *Tinospora cordifolia*, *Withania somnifera*, *Centella asiatica* , *Azadiracta indica* and its individual herb extracts were separately screened for the antibacterial activity by measuring zone of inhibition. Antibacterial activity was compared with standard Gentamycin disc (10mcg/disc) on three Gram negative and two Gram positive bacteria namely, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella tryphyimurium*, *Staphylococcus aureus* and *Bacillus subtilis* by Agar well-diffusion assay method. Mueller-Hinton agar was used as the medium for the antibacterial activity. Study revealed significant synergistic antibacterial activity of alcoholic polyherbal extract when compared to its individual herb extract. Alcoholic extract of the polyherb showed better antibacterial activity than its aqueous extract. This confirms the objective of the study to develop a polyherbal pharmaceutical formulation which can produce a desired pharmacological effect with lesser concentrations of the herbal ingredients than a pharmaceutical formulation with the same individual ingredient for the same pharmacological effect.

Keywords: Antibacterial activity, inhibition, diffusion, Poly herbal formulation.

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INRODUCTION

Herbal drugs constitute a major part in all the traditional systems of medicine. With the emerging interest in the world to adopt and study the traditional system and to exploit their potentials based on different health care systems, the evaluation of the rich heritage of the traditional medicine is essential [1]. Now-a -days, the synthetic drugs are although dominating the market but the element of toxicity that these drugs entail, cannot be ruled out[2]. Herbal drugs are tremendously explored for anti-infective compounds as the emergence of multidrug resistant bacteria are prevalent. The present research focuses on the development of a new herbal formulation with maximum antibacterial activity and minimum resistance and toxicity. The polyberbal extract contained four different crude drugs namely Stems of *Tinospora cordifolia* (Menispermaceae), Roots of *Withania somnifera* (Solanaceae), *Whole plants of Centella asiatica* (Umbelliferae), and Leaves of *Azadiracta indica* (Meliaceae).

MATERIAL AND METHODS

Crude drug

- Stems of *Tinospora cordifolia,*
- Roots of *Withania somnifera ,*
- Whole plants of *Centella asiatica,*
- Leaves of *Azadiracta indica*

Polyherb: Equal proportion of coarsely powdered crude drug

Plant Extracts

- Aqueous extract of Polyherbs
- Alcoholic extract of Polyherbs
- Alcoholic extract of *Tinospora cordifolia*
- Alcoholic extract of *Withania somnifera*
- Alcoholic extract of *Centella asiatica*
- Alcoholic extract of *Azadiracta indica*

Microorganism used: *Escherichia coli(*MTCC727), *Klebsiella pneumonia (MTCC)*, *Salmonella tryphyimurium*(MTCC98), *Staphylococcus aureus* (MTCC3160) and *Bacillus subtilis*(MTCC3053)

Standard drug used: Gentamycin 100µg disc

Media: Mueller-Hinton agar

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METHOD

Extraction

Plant extracts were prepared by Soxhlet extraction.

Standardization

- Physical parameters : Colour, odour, consistency (Total solids),pH of the crude extract (aqueous and alcoholic) was standardized as shown in table no.1.

- Phytochemical screening [3,4]:The crude plant extracts of polyherb was subjected to qualitative phytochemical screening by standard procedure .Results are shown in table no 2.

Table 1: Physical parameters

Parameter	Aqueous extract of Polyherbs	Alcoholic extract of Polyherbs
Colour	Blackish brown	Blackish green
Odour	Characteristic	Characteristic
Consistency(total solids)	71.64%w/w	63.72%w/w
pH (1%w/v)	4.5	3.65
Yield(w/w)	8.53%	12.6% w/w

Table 2: Phytochemical screening

S1.No	Qualitative Test	Aqueous Extract of Polyherb	Alcoholic Extract of Polyherb
1	Alkaloids	Absent	Present
2	Sterols	Absent	Present
3	Triterpenoids	Present	Absent
4	Saponins	Present	Present
5	Tannins	Present	Present
6	Flavones& Flavanoids	Absent	Present
7	Aminoacids	Present	Absent
8	Carbohydrate	Present	Present
9	Fixed Oils	Absent	Present

Table 3: Results of Screening of Antibacterial Activity

Microorganism	Aqueous Extract of Polyherb(150µl) (100µg/ml)		Alcoholic Extract of Polyherb(150µl) (100µg/ml)		Positive Control (Gentamycin) (100µg/disc)	
	ZI (mm)±SD	Inference	ZI (mm)±SD	Inference	ZI(mm)	Inference
Escherichia coli	12±0.7	R	15±0.5	S	29±0.5	S
Klebsiella pneumoniae	11±0.7	R	15±0.5	S	27±0.5	S
Salmonella tryphyimurium	13±0.5	I	15±0.5	S	29±0.5	S
Staphylococcus aureus	12±0	I	17±0.7	S	28±0.6	S
Bacillus subtilis	14±0.3	R	20±0.7	S	29±0.5	S

ZI- Zone of inhibition(mm) n=3, SD=Standard deviation

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Table 4: Results of Screening of Antibacterial Activity

Microorganism	Alcoholic Extract of Polyherb(150µl) (100µg/ml)		Alcoholic extract of *Tinospora cordifolia*		Alcoholic extract of *Withania somnifera*		Alcoholic extract of *Centella asiatica*		Alcoholic extract of *Azadiracta indica*		Positive Control (Gentamycin 100µg)		Negative Control	
	ZI (mm)±SD	Inference	ZI (mm)±SD	Inference	ZI (mm)	Inference	ZI (mm)±SD	Inference	ZI (mm)±SD	Inference	ZI (mm)±SD	Inference	ZI (mm)±SD	Inference
Escherich-ia coli	15 ±0.5	S	13±0.3	I	21 ±0.5	S	18 ±0.5	S	15 ±0.5	S	29 ±0.2	S	0	R
Klebsiella pneumoniae	15 ±0.5	S	13±0.5	I	20 ±0.3	S	17 ±0.2	S	17 ±0.5	S	27 ±0.3	S	0	R
Salmonella tryphyimurium	15 ±0.5	S	15±0.2	S	21 ±0.2	S	17 ±0.3	S	15 ±0.3	S	29 ±0.2	S	0	R
Staphylococcus aureus	17 ±0.7	S	15±0.6	S	20 ±0.5	S	18 ±00	S	15 ±0.5	S	28 ±0.5	S	0	R
Bacillus subtilis	20 ±0.7	S	13±0.5	I	20 ±0.5	S	16 ±0.5	S	18 ±0.5	S	29 ±00	S	0	R

ZI- Zone of inhibition(mm) n=3, SD=Standard deviation

Antibacterial activity

Agar well diffusion assay was used for the determination of antibacterial activity. 18 hours incubated bacterial cultures were swabbed evenly on the Mueller- Hinton agar plates using sterile swabs[5-9]. Wells (0.7cm diameter) were made in the agar using sterile cork borer.150µl of plant extracts (100 µg/ml) was added to the well using micro pipette and sterile tips. Gentamycin 100µg per disc was placed as the positive control. The plates were kept for 2hrs undisturbed for complete diffusion of the extract. Then the plates were incubated at 37^{o}c for 24 hrs. After 24hrs, the zone of inhibition was checked using Hi Media zone size checking scale. The method was repeated thrice (n=3) and standard deviation was calculated as indicated in table No3, 4.

Hi Media zone size checking scale

ZONE SIZE IN mm	INFERENCE
0-12	R-Resistant
13-14	I-Intermediate
15- above	S-Sensitive

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RESULT AND DISCUSSION

Preliminary Phytochemical screening of crude aqueous extract of the polyherb showed the presence of Triterpenoids,Tannins ,Saponins,carbohydrates and aminoacids.Crude alcoholic extract showed the presence of Alkaloids, Sterols, Tannins, Saponins, Flavones & flavanoids, carbohydrates and fixed oil. Antibacterial screening showed that alcoholic extract of polyherb is having better activity than its aqueous extract. On comparison of individual Alcoholic extracts *Withania somnifera* showed best activity. Study revealed significant synergistic antibacterial activity of alcoholic polyherbal extract when compared to its individual herb extract.

CONCLUSION

This confirms the objective of the study to develop a polyherbal pharmaceutical formulation which can produce a desired pharmacological effect, with lesser concentrations of the herbal ingredients than a pharmaceutical formulation with the same individual ingredient for the same pharmacological effect.

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