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ASSESSMENT OF ACTIVE COMPONENTS FROM INVASIVE ALIEN PLANT SPECIES DISTRIBUTED IN BIHAR

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ABSTRACT

Plant pathogenic microbes are on rise and thus causing plants quality deterioration. Use of chemicals to lessen their effect, knowingly or unknowingly leaves their side effects. Natural product, a suitable alternative as antimicrobial agent to curb effect of such plant pathogenic microbes has led to study many rare and uncommon plants. Our study aimed to find some active constituents from invading alien species (IAS) predominant in Bihar region. *Ageratum conyzoides* (L.) L, *Parthenium hysterophorus* L., *Eupatorium adenophorum* Hort. Berol.ex Kunth, *Galinsoga parviflora* Cav. and *Mikania micrantha* (L.) Willd were chosen to study their active constituents using qualitative phytochemical analysis and GC-MS spectra. The extracts were evaluated to study their antimicrobial effect against a plant pathogenic bacteria (*S.aureus*) and fungus (*F.solani*). The most significant results were observed in the case of *Ageratum conyzoides* L. extract (ACE) and *Parthenium hysterophorus* L. extract (PHE) against *S.aureus* and moderate activities against fungus by *A.conyzoides* (L.) L extract, *E.adenophorum* Hort. Berol. ex. Kunth extract and *M.micrantha* (L.) Willd extract. Thus these plants may be promising source as new antimicrobial drugs due to its efficacy.

Keywords: Invasive alien species, Biodiversity, Chromatogram, Retention time, Soxhlet, Inhibition, Phytochemicals

INTRODUCTION

Invasion of Flora species has majorly increased in past few years due to Globalization and rapid changes in natural habitats. The effect of these invasive species has caused reduction in the discreteness among flora and fauna of various regions. The percentage of non-indigenous species over native species in continental and island are reported to be 20% and 50% respectively. Few advantageous properties which IAS possess over native species are- faster growth rate and more production of biomass as compared to native species, greater competitive capability, high reproductive efficiency with properties like large number of seeds, productive dispersal, vegetative reproduction, rapid establishment and other traits that help them adapt to new habitats **[1, 2]**. IAS also have potential to survive in extreme conditions and allelopathic in nature **[2, 3]**. Invasive success is a combined result of autecological attributes of invading species as well as biotic and abiotic properties of the target habitat **[4]**.

Antimicrobial agents are of great interest to the researchers in present scenario as microbes are gradually becoming resistant to the existing agents especially antibiotics. There is immediate need of agents which possess broad spectrum antimicrobial potential against human or plant pathogenic species. Therefore, researchers are looking plant as one of the best possible source for such agents. Plant produces antimicrobial products through process of secondary metabolism as defense mechanism to protect themselves from pathogenic attack **[5]**. These product are eco-friendly and comparatively cheaper thus attracting more researcher to explore their antimicrobial properties **[5-7]**. Thus the secondary metabolites produced by plants which are not harmful and very specific in nature can proved to be of great importance for human health. Our study is to find such antimicrobial agents which could be potential alternatives to normal marketed bactericidal agents.

Due to increasing trade and transcontinental transport, the floras of Indian subcontinent namely, upper Gangetic plain **[8],** Ranchi **[9]** and Allahabad **[10],** have a number of Invasive Alien Species from various parts of the world. About 8% of the Indian flora constitutes aliens of which 55% are American, 30% Asian and Malaysian and 15% European and Central Asian species **[11]**. As these plant species have a negative impact on the environment and biodiversity because of their speedy invading property, they tend to produce products that curb the spread of their competitors. We aim to find some active

components from few alien species which would prove beneficial against some plant pathogenic bacteria and fungus to suppress their growth.

MATERIAL AND METHODOLOGY

Collection of Plants and identification

Ageratum conyzoides (L.) L, *Parthenium hysterophorus* L., *Eupatorium adenophorum* Hort.Berol. ex Kunth, *Galinsoga parviflora* Cav. and *Mikania micrantha* (L.) Willd samples were collected from their natural habitat between March and April, 2019 from the district Gopalganj, Bihar, India. The samples were collected in plastic zipped pouch and brought to the laboratory. Whole plant of each species was washed thoroughly in running water, followed by rinsed with distilled water and then shade dried for 15 days at room temperature and powdered in an electric blender. Identification of the each species was authenticated by a plant taxonomist and each plant specimen was submitted to the herbarium of Department of Botany, Gopeshwar College, Hathwa, Gopalganj, Bihar.

Extraction

Extraction was done by successive soxhlet extraction using solvents in increasing order of polarity *viz*. petroleum ether, ethyl acetate and methanol **[12]**. 25gm of each of the powdered plant was extracted successively with petroleum ether, ethyl acetate and methanol for 7 hours. The filtrates obtained from soxhlet were evaporated to dryness in a rotary evaporator under reduced pressure. Dried powders of extracts were kept in sterile bottles at 4°C in a refrigerator until used for analysis. The powdered samples were dissolved immediately before use in 10% DMSO to make four different concentrations of 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml

Primary screening for Phytochemical compounds

The presence of alkaloids, resins, saponins, glycosides, tannins, flavonoids, cardiac glycoside, steroidal ring, steroidal terpenes, anthraquinone and carbonhydrates were determined as described by **[13-19].**

Active component Analysis using GC-MS

Determination of Phytochemical compound profiles present in the extracts of *Ageratum conyzoides* (L.) L, *Parthenium hysterophorus* L., *Eupatorium adenophorum* Hort.Berol. ex Kunth, *Galinsoga parviflora* Cav.and *Mikania micrantha* (L.) Willd were analyzed using GC-MS Analysis equipment (Thermo Scientific Co.) Thermo GC-TRACE ultra ver.: 5.0, Thermo MS DSQ II. The conditions followed for experimental set up of GC-MS system were as follows: TR 5-MS capillary standard polar column, dimension: 30Mts, ID: 0.25 mm, Film thickness: 0.25µm. Flow rate of mobile phase (carrier gas: He) was set at 5.0 ml/min. In the gas chromatography part,

temperature programme (oven temperature) was 40°C raised to 350°C at 5°C/min and injection volume was 1 µl. Samples dissolved in methanol were run fully at a range of 50-650 m/z and the results were compared by using Wiley Spectral Library Search Programme.

Test Organism and Culturing of Isolates:

Staphylococcus aureus (ATCC 29213) and *Fusarium solani* (ATCC 201839) organisms were used in the study to evaluate the antibacterial activity of extracts of *Ageratum conyzoides* (L.) L, *Parthenium hysterophorus* L.,*Eupatorium adenophorum* Hort.Berol. ex Kunth, *Galinsoga parviflora* Cav.and *Mikania micrantha* (L.) Willd. Bacterial and Fungal strains were maintained on Nutrient agar and Potato Dextrose Agar Plates respectively. Cultures were incubated overnight at 37°C and 25°C before use. The stock cultures for two isolates were maintained as glycerol stock at -70°C for any future use.

Antibacterial and Antifungal Activity

The inhibitory actions of 5 plants were performed and analyzed by Disk Diffusion Method given by **Heately (1944) [20]** method. *Pseudomonas fluorescens* (ATCC 13525) and *Aspergillus niger* (ATCC 16404) were grown in Mueller Hinton broth (MHB) at 37°C for 24 hours in a shaker incubator. The incubated bacterial suspension and grown fungal spores were taken 0.1ml and spread on Muller Hinton Agar Media Plates. The suspension spread on agar plates were then left to be absorbed on the surface of media, disks soaked with variable concentrations (12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml) of plant extract were then placed on media at equal distant. The bacterial and fungal plates were then incubated at 37°C and 25°C respectively for 24 (bacteria) and 72 hours (fungus). The clear zone around the disk (zone of inhibition) was recorded in mm and compared with standard antibiotics.

RESULT AND DISCUSSION

The work screens five invasive alien species *Ageratum conyzoides* (L.) L, *Parthenium hysterophorus* L., *Eupatorium adenophorum* Hort.Berol. ex Kunth, *Galinsoga parviflora* Cav.and *Mikania micrantha* (L.) Willd. for presence of common phytochemical compounds using standard methods of qualitative phytochemical analysis and Gas Chromatography Mass Spectrometry technique. The phytochemicals screened for the analysis were alkaloid, flavonoid, glycoside, saponin, tannin, terpenoid, sugar and anthroquinone. Extracts were prepared by successive soxhlet extraction method using solvents in increasing order of polarity i.e., petroleum ether, ethyl acetate and methanol. The method as described earlier (**Cowan, 1999) [12]** solubilizes and extracts most of the phytochemicals. The

results are shown **(Table 1)** *Ageratum conyzoides* (L.) L, extract showed presence of glycosides, flavonoids, saponins, tannins, terpenoids and phenols and absence of alkaloid and anthroquinone. *Parthenium hysterophorus* L. showed presence of only two constituents' terpenoids and phenol while absence of rest of the components. *Eupatorium adenophorum* Hort. Berol. ex Kunth showed presence of saponin, tannin, terpenoid and phenol while absence of rest of phytochemicals. *Galinsoga parviflora* Cav. had revealed presence of alkaloid and sugars. *Mikania micrantha* (L.) had presence of glycoside, terpenoid and phenol.Already reports work on phytochemical screening of these plants are **[21-24].**

The GC-MS analysis of crude and active band as shown in Figures **(Figure 1 & 2)** for *Ageratum conyzoides* (L.) Lextract (ACE) and *Parthenium hysterophorus* L. extract (PHE) indicates the presence of different components. As these two plant showed maximum (significant) inhibitory action against test organisms in this study, therefore phytochemical compound profile were analyzed for them. The major components present in the *Ageratum conyzoides* (L.) L extract were propanephosphonic acid, bis(trimethylsilyl) ester (RT:10.83min), Fluoren-9-ol, 3,6-dimethoxy-9-(2-phenylethenyl) (RT:11.19min), Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl (RT:14.28 min), 6,8-Difluoro-2,2,4,4,6,7,7,8,9,9-decamethyl-[1,3, 5,2,4,6,7,8,9] trioxahexasilonane (RT:16.18 min), 5-hydroxy-7-methoxyflavanone, tert.-butyldimethylsilyl ether (RT: 16.35 min), Hexadecanoic acid, ethyl ester (RT: 23.49 min), Linoleic acid ethyl ester (RT:29.46 min), Ethyl Oleate (RT:29.64 min) and Bis[di(trimethylsiloxy)phenylsiloxy]trimeth ylsilox yphenylsiloxane (RT:29.75 and 34.9 min) **(Table 2)**.

The major components present in the *Parthenium hysterophorus* L. extract (PHE) were Tridecanoic acid, 4,8,12-trimethyl-, methyl ester (RT: 10.69 min), Octadecane,3-ethyl-5-(2-ethylbutyl)- (RT: 10.9 min, 10.9 min, 11.52 min, 11.7 min, 11.89 min, 12.72 min), Fumaric acid, 2-ethylhexyl tridec-2-yn-1-yl ester (RT:12.94min), cis-5,8,11,14,17-Eicosapentaenoic acid (RT: 13.82 min), Octadecane, 3-ethyl-5-(2-ethylbutyl)- (RT: 14.17 min), Methyl glycocholate, 3TMS derivative (15.87 min). The Mass Spectrometer determined the compounds which eluted at variable times to and helped to identify the structure and properties of the compounds. These mass spectra are fingerprint of that compound which can be identified from the data library. Thus identified compounds can be further purified and used for further analysis to get

deep insight into their role and usage **(Table 3)**.

The antibacterial Activity of the extracts obtained from five different plants were tested by Disk Diffusion Method **[26].** The obtained powders of the extract were dissolved in DMSO just before the assay. Serial dilutions were performed initially in range of concentration (11.5 µg/µl- 750 µg/µl) to find out the suitable concentration for the assay. The extract concentration used for each plant was 187 µg/µl dissolved in DMSO. The zones inhibition of mean diameter of the plant extract against the tested bacteria ***Staphylococcus aureus*** **(ATCC 29213)** and fungus ***Fusarium solani*** **(ATCC 201839)** are tabulated in **Table 4**.

The antimicrobial activity of *Ageratum conyzoides* (L.) L, *Parthenium hysterophorus* L., *Eupatorium adenophorum* Hort.Berol. ex Kunth, *Galinsoga parviflora* Cav.and *Mikania micrantha* (L.) Willd extracts showed variable results. Earlier inhibitory actions of these alien species have been reported against some pathogenic bacteria and fungus. The aim of this study is to assess the antimicrobial activity possessed by these plants against plant pathogenic microbes. As these alien species have highly invading property, therefore they have potential to produce compounds which could inhibit other pathogenic microbes. As they easily grow with faster rate, therefore a rich source could be obtained from them. In our study, *Galinsoga parviflora* Cav. extract (GPE) showed lower activity against *S.aureus* (18.00±0.00) and no activity against *F.solani* (0.00±0.00), *Mikania micrantha* (L.) Willd extract (MME) against *S.aureus* (16.67±0.58) and *F.solani* (11.33±1.1547). Moderate inhibitory action was observed by *Eupatorium adenophorum* Hort. Berol. ex Kunth extract (EAE) (*S.aureus*: 24.67±0.58 and *F.solani:*14.33±2.516). The most significant results were observed in the case of *Ageratum conyzoides* (L.) L extract (ACE) and *Parthenium hysterophorus* L. extract (PHE) with comparatively similar zone of inhibition of 28.33±0.58 and 30.33±0.58 respectively, as that of positive control (31.67±0.58) against *S.aureus*. In case of antifungal, not very significant inhibitory actions were observed with low to moderate activities in all the plants *Ageratum conyzoides* (L.) L extract (12.67±1.155), *Parthenium hysterophorus* L. extract (8.33±0.577), *Eupatorium adenophorum* Hort.Berol. ex Kunth extract (14.33±2.516), *Galinsoga parviflora* Cav. extract (0.00±0.00) and *Mikania micrantha* (L.) Willd extract (11.33±1.1547) **Figure 3**.

Table 1: Phytochemical profile of Tested plant extracts

Phytochemical screen								
Extract	**Alkaloid**	**Flavonoid**	**Glycosides**	**Saponins**	**Tannins**	**Terpenoids**	**Phenols**	**Anthroquinone**
ACE	**A**	**P**	**P**	**P**	**P**	**P**	**P**	**A**
PHE	**A**	**A**	**A**	**A**	**A**	**P**	**P**	**A**
EAE	**A**	**A**	**A**	**P**	**P**	**P**	**P**	**A**
GPE	**P**	**A**	**A**	**A**	**A**	**A**	**P**	**A**
MME	**A**	**A**	**P**	**A**	**A**	**P**	**P**	**A**

A= Absent; P=Present; ACE: A.conyzoides; PHE: P.hysterophorus: EAE: E.adenophorum: GPE: G.parviflora; MME:M.micrantha

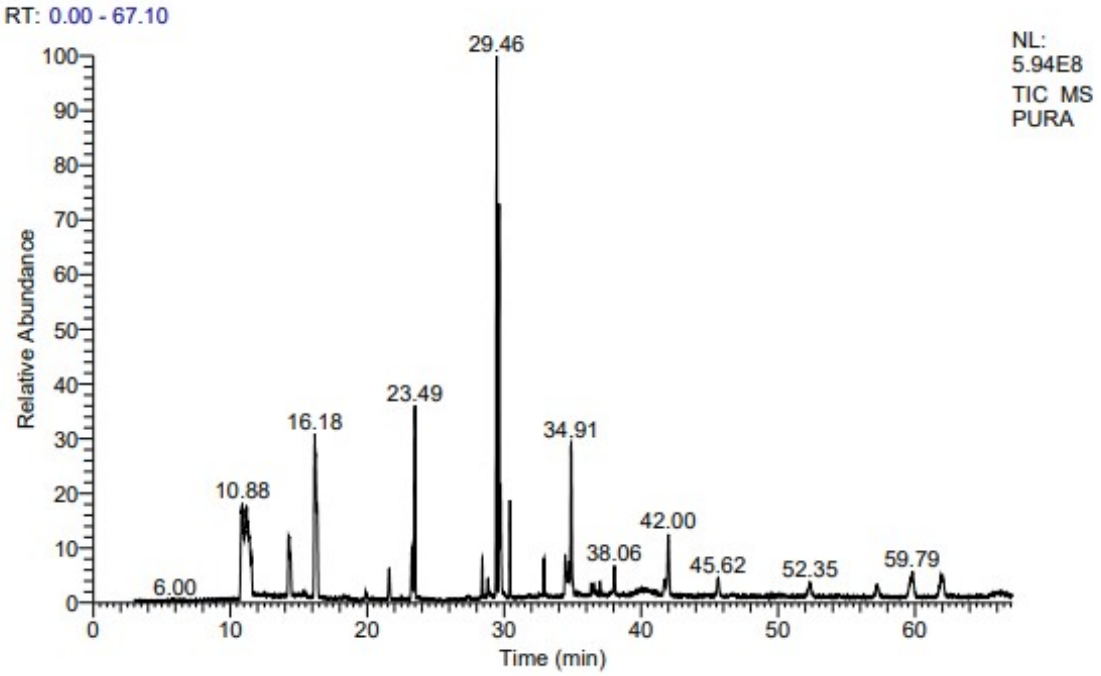

Fig 1: GC-MS Chromatogram of Ageratum conyzoides extract

Table 2: Bioactive compounds identified using GC-MS in whole plant extract of *Ageratum conyzoides* (ACE)

RT	Scan#	Probability	Compound name	Molecular Formula	Molecular Weight
10.83	**6502.0000 00**	**11.62**	**Propanephosphonic acid, bis(trimethylsilyl) ester**	**$C_{10}H_{22}O_4Si_2$**	**262.45 g/mol**
11.19	**6717**	**22.74**	**Fluoren-9-ol, 3,6-dimethoxy-9-(2-phenylethenyl)-**	**$C_{23}H_{20}O_3$**	**344.4 g/mol**
14.28	**8566.0000 00**	**32.94**	**Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl**	**$C_{14}H_{42}O_6Si_7$**	**503.07 g/mol**
16.18	**9707.0000 00**	**25.02**	**6,8-Difluoro-2,2,4,4,6,7,7,8,9,9-decamethyl-[1,3,5,2,4,6,7,8,9]trioxahexasilonane**	**$C_{10}H_{30}F_2O_3Si_6$**	**404.85 g/mol**
16.35	**9810.0000 00**	**27.38**	**5-hydroxy-7-methoxyflavanone, tert.-butyldimethylsilyl ether**	**$C_{22}H_{28}O_4Si$**	**384.5g/mol**
23.49	**1496.0000 00**	**75.97**	**Hexadecanoic acid, ethyl ester**	**$C_{18}H_{36}O_2$**	**284.5g/mol**
29.46	**1765.0000 00**	**44.9**	**Linoleic acid ethyl ester**	**$C_{20}H_{36}O_2$**	**308.5g/mol**
29.64	**17784.0000 00**	**29.37**	**Ethyl Oleate**	**$C_{20}H_{38}O_2$**	**310.5g/mol**
29.75	**17848.0000 00**	**37.88**	**Bis[di(trimethylsiloxy)phenylsiloxy]trimethylsiloxyphenylsiloxane**	**$C_{33}H_{60}O_7Si_8$**	**793.5g/mol**
34.9	**20939.0000 00**	**95.07**	**Bis[di(trimethylsiloxy)phenylsiloxy]trimethylsiloxyphenylsiloxane**	**$C_{33}H_{60}O_7Si_8$**	**793.5g/mol**

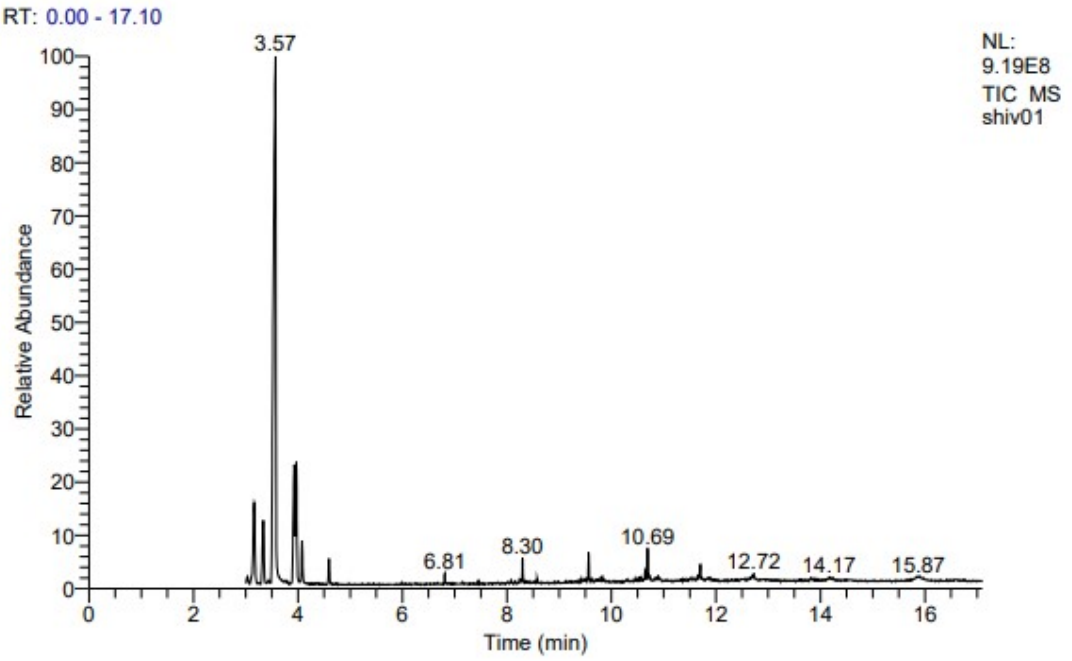

Figure 2: GC-MS Chromatogram of *Parthenium hysterophorus* extract

Table 3: **Bioactive compounds identified using GC-MS in whole plant extract of *Parthenium hysterophorus (PHE)***

RT	Scan#	Probability	Compound name	Molecular formula	Molecular Weight
10.69	2295.0000 00	12.54	Tridecanoic acid, 4,8,12-trimethyl-, methyl ester	$C_{17}H_{34}O_2$	270.5g/mol
10.9	2356.0000 00	17.69	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.7g/mol
11.52	2542.0000 00	17.43	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.7g/mol
11.7	2595.0000 00	8.48	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.7g/mol
11.89	2651.0000 00	23.27	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.7g/mol
12.72	2900.0000 00	13.13	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.7g/mol
12.94	2964.0000 00	11.29	Fumaric acid, 2-ethylhexyl tridec-2-yn-1-yl ester	$C_{25}H_{42}O_4$	406.6g/mol
13.82	3228.0000 00	12.41	cis-5,8,11,14,17-Eicosapentaenoic acid	$C_{20}H_{30}O_2$	302.5g/mol
14.17	3332.0000 00	20.28	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.7g/mol
15.87	3838.0000 00	28.97	Methyl glycocholate, 3TMS derivative	$C_{36}H_{69}NO_6Si_3$	696.2g/mol

Table 4: Antibacterial *(P.aeruginosa)* and Antifungal *(C.albicans)* activity of 5 plant extracts

S.No.	Plant extract	Sample Code	Zone of Inhibition (mm)±S.D.	
			Staphylococcus aureus (ATCC 29213)	*Fusarium solani (ATCC 201839)*
1	*Ageratum conyzoides*	ACE	28.33±0.58	12.67±1.155
2	*Partheniumhysterophorus*	PHE	30.33±0.58	8.33±0.577
3	*Eupatorium denophorum*	EAE	24.67±0.58	14.33±2.516
4	*Galinsoga parviflora*	GPE	18.00±0.00	0.00±0.00
5	*Mikania micrantha*	MME	16.67±0.58	11.33±1.1547
6	*Positive control*	PC	31.67±0.58	28.33±1.1547
7	*Negative control*	NC	0.00±0.00	0.00±0.00

***Positive control:0.2 mg/ml Ciprofloxacin; Negative control:DMSO (100%); Zones include size of the well (6mm)**

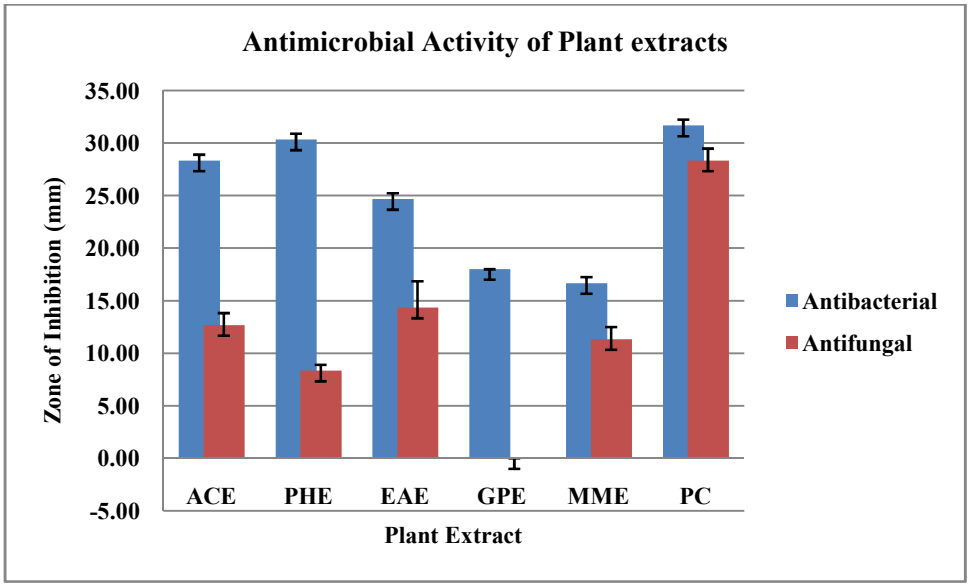

Fig 3: Comparison of Antibacterial and Antifungal Activities possessed by Plant Extracts

Our results were in accordance to **Nair and Chanda (2006)** work where also *Parthenium hysterophorus L.* leaves extract exhibited significant activity against all the human pathogens studied against *Staphylococcus sp., Klebsiella sp., Pseudomonas sp., Proteus sp., E. coli, Enterobacter sp., Streptococcus sp., and Citrobacter sp*. and the results observed for antifungal activities, **Jyotilakshmi *et al.*, (2017) [28]** reported similar results on *Mikania micrantha* (L.) Wild extract as potent antidermatophytes. We found *Eupatorium adenophorum* Hort. Berol. ex Kunth extract (14.33±2.516)and *Mikania micrantha* (L.) Wild extract (11.33±1.1547) as best antifungal agents.

CONCLUSION

Invasive alien species develop properties which help them to sustain in adverse and non-native environment. These properties help them to dominate over the native species, thus making them abundantly available throughout India. Our study aims to use the properties of five such invading alien species majorly common in Bihar region to produce some beneficial products out of it. The negative impacts of IAS are well known to everyone, but the positive side is yet to be explored. The study is an attempt to find some active constituents which posses' antimicrobial property against plant pathogenic microbes. Natural sources for such antimicrobial agents are easy to access, cheap and have least side effects on plants on applying to them. Further detailed studies on other plant pathogenic microbes and elucidation of some biological activities will be highly useful and are on the way.

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REFERENCES

[1] Simberloff, D., Parker, I.M and Windle, P.M.2005. Introduced species policy, management and future research needs. *Front. Ecol. Environ.,* 3; pp. 12-20.

[2] Sharma, G.P., Singh, J.S and Raghubanshi, A.S. 2005. Plant invasions: emerging trends and future implications. *Curr. Sci.,*88; pp. 726-734.

[3] Huang, Q.Q., Wu, J.M., Bai, Y.Y, Zhou, L and Wang, G.X.2009. Identifying the most noxious invasive plants in China: role of geographical origin, life form and means of introduction. *Biodivers. Conserv.*18; pp. 305-316.

[4] Higgins, S.I and Richardson, D.M.1996. A review of models of alien plant spread. *Ecol. Model.,* 87; pp. 249-265.

[5] Macdonald, M.M.2018. Evaluation of Alien Invasive weedy plants for activity against plant pathogenic fungi. M.Sc. Thesis, University of Pretoria, South Africa.

[6] Rhouma, A., Daoud, H.B., Ghanmi, S., Salah, H.B., Romdhane, M and Demak, M. 2009. Antimicrobial activities of leaf extracts of Pistacia and Schinus species against some plant pathogenic fungi and bacteria. *J. Plant Pathol.,* 91; pp. 339-345.

[7] Abera, A., Lemessa, F and Muleta, D. 2011. The antifungal activity of some medicinal plants against coffee berry disease caused by *Colletotrichum kahawae*. Int. J. Agric. Res. 6; pp. 268-279.

[8] Raizada, P. 2006. Ecological and vegetative characteristics of a potent invaders *Hyptis suaveolens* Poit. from India. *Lyonia*., 11; pp. 115-120.

[9] Maheshwari, J.K and Paul, S.R.1975. The Exotic flora of Ranchi. J Bombay *Nat. Hist Soc.,* 72; pp.158-188.

[10] Sharma, B.D. 1984. Exotic Flora of Allahabad, Botanical Survey of India, Dehradun.

[11] Nayar, MP. 1997. Changing patterns of *Indian flora. Bull. Bot. Surv. India.,* 19; pp.145-154.

[12] Cowan, M.M.1999. Plant products as antimicrobial agents. *Clin Microbiol Rev.*12; pp. 564-82.

[13] Wall, M.E., Eddy, C.R., Clenna, M.L and Klump, M.E.1952.

Detection and Estimation of Steriod Sapogenina in Plant Tissue. Anal. Chem., 241; pp.1337-1349.

[14] Kapoor, L.D., Singh, A., Kapoor, S.L and Strivastava, S.N.1969. Survey of Indian Medicinal Plants for Saponins Alkaloids and Flavonoids. *Lloydia*, 32;pp. 297-302.

[15] Harborne, J.B.10984. Methods of plant analysis. In Phytochemical methods. *Springer*, Dordrecht, pp. 1-36.

[16] Sofowora A.1982. Medicinal Plants and Traditional Medicine in Africa. John Wiley, *Chinchester*, pp. 179-187.

[17] Trease, G.E and Evans, W.C.1989. Pharmacognosy. 13th edition. Bailliere Tindall Ltd. London.

[18] Akinyemi, K.O., Oladapo, O., Okwara, C.E., Ibe, C.C and Fasure, K.A2005. Screening of Crude Extracts of Six Medicinal Plants Used in South-West Nigeria unorthodox medicine anti-methicillin resistant *Staphylococcus aureus* activity. BMC Complementary and Alternative Medicine, 5; pp. 1-7.

[19] Junaid, S.A, Olabode, A.O., Onwuliri, F.C, Okwori, A.E.J and Agina, S.E.2006. The antimicrobial properties of *Ocimum gratissimum* extracts on some selected bacterial gastrointestinal isolates. *Afr. J. of Biotechnology,* 5;pp. 2315-2321.

[20] Heatley, N.G. 1944. A method for the assay of penicillin. *Biochemical Journal,* 38; pp. 61-78.

[21] Krishnaveni, M., and Dhanalakshmi, R.2014. Phytochemical analysis of *Parthenium hysterophorus* L. leaf. *World Journal of Pharmaceutical Research,* 3; pp. 1066-1074.

[22] Siddiqui, S., Verma, A., Rather, A.A., Jabeen, F and Meghvansi, M.K. 2009. Preliminary phytochemical analysis of some important medicinal and aromatic plants. *Advance Biological Research,* 3; pp. 188-195.

[23] Chitravadivu, C., Manian, S and Kalaichelvi, K. 2009. Qualitative analysis of selected medicinal plants, Tamil Nadu, India. *Middle East Journal of Scientific Research,*4; pp. 144-146.

[24] Ashok Kumar, P., Rajkumar., and Kanimozhi, M.2010. Phytochemical screening and antimicrobial activity from five Indian medicinal plants against human pathogens. *Middle East*

Journal of Scientific Research, 5; pp. 157-162.

[25] Savithramma N, Linga Rao M and Suhrulatha D.2011. Screening of medicinal plants for secondary metabolites. *Middle East Journal of Scientific Research,* 8; pp. 579-584.

[26] Srinivasan, D., Nathan, S., Suresh, T and Lakshmana P. 2001. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine, *Journal of Ethnopharmacology,* 74; pp. 217-220.

[27] Nair, R and Chanda, S.2006.Activity of some mecicinal plants against certain pathogenic bacterial strains. *Indian J Pharmacol.* 38; pp. 142-144.

[28] Jyothilakshmi, M., Jyothis, M., Narayanan, G.N.H and Latha, M.S.2017. Antidermatophytic and protease-inhibiting activities of zerumbone: A natural sesquiterpene from the rhizome of *Zingiber zerumbet* (L.) Roscoe ex. JE; Smith. *Pharmacognosy magazine*, 13; pp. 2-13.