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Synergistic Effect Of Combined Antibiotic And *Asparagus Racemosus* Bark Extract As A Potent Antimicrobial Activity

Namrata Patel[1*], Niharika Jegoda[2], Kinjal Patel[3], Piyush J. Vyas[4]

[1*,3,4]Department of Chemistry, Sheth M. N. Science College, Patan-384265, Gujarat, India.
[2]Department of Chemistry, Gokul Science College, Sidhpur-384151, Gujarat, India.
E-mail: vyaspiyushj@yahoo.com

ABSTRACT
Antimicrobial drugs derived from plants alone cannot protect against diseases due to some limitations against bacterial growth in the environment. Research has consistently shown thatantibiotics derived from plants are synergistic; this me ans that although they contain fewer
antibiotics when taken alone, they make the drug more effective when combined with medication. Antibiotics and herbal extracts work synergistically to prevent drug resistance, opening new avenues for treating infectious diseases. The synergistic effect of plant extracts and antibiotics was analyzed using the agar positive diffusion method. According to the research results, the activity level was higher when Asparagus racemosa plant extract was combined with antibacterial drugs. The results of this study suggest that combining plant extracts with antibiotics may be beneficial for newly developed antibiotics. According to HPTLC data, the combination of antibiotics with antibiotics do es not lead to new compounds.

KEYWORD: *Asparagus racemosus*, HPTLC, Antibiotics, Antimicrobial Activity

INTRODUCTION

The genus Asparagus consisted of around 300 species around the world, out of which 25 species are chronicled in India. *A. racemosus* is generally distributed across the sphere and its circulation ranges from tropical Africa, Java, Australia, Sri Lanka, Southern parts of China and India, but it is mainly cultivated in India.[1-6]

The *A. racemosus* is an imperative medicinal plant which is observed as a 'rasayana' which means plant drugs endorsing general well-being by swelling cellular vitality and battle.[7] Use of *A. racemosus* is declared in the ancient poetry of Ayurveda. Conventionally, A. racemosus is showed in epilepsy, vata sicknesses, brain tonic, helps in changeable cardiac sicknesses and hypertension.[8,9]

It is lengthily used in male genital dysfunctions, oligospermia, spermatogenic irregularities and other male sicknesses such as painfull micturition. It is also discovered in Ayurvedic inventions for digestive distress, indigestion, amoebiasis, piles and feebleness.[10] In females, settled by the doctors in habitual abortions, feebleness of the uterus, excessive bleeding through menstruation. Topical reports and experiments released Shatavari as antidiarrhetic, antispasmodic, aphrodisiac, antidysenteric, demulcent, diuretic, galactagogue, nutritive, mucilaginous, refrigerant, stomachic goods and works as a tonic for human beings.[11-13] It is also known to underpin the safe system and protect vital publications like heart, brain and other organs of the body. This review is a argument about the agronomy, morphology, phytochemistry, biological happenings, and safety silhouette and conservation systems for this herbal.[14,15]

Ayurveda defines Asparagus racemosus as rasayana and galactogogue, which is secondhand to treat various sicknesses such as ulcer, dyspepsia and frailty. It contains adventitious root system with tuberous roots.[16] These tuberous roots after suitable processing and ventilation are used as ayurveda medicine, with the name of Shatavari. Its leaves are condensed to form cladodes. Branches contain spines on them. In Indian medicine it is well known as an antispasmodic, aphrodisiac, demulcent, diuretic, galactogogue and refrigerant. It is also used in the treatment of diarrhea, rheumatism, diabetes and brain complaints.[17] During earlier investigations influence of nourishments on growth and biochemical conformation and in vitro proliferation of Asparagus racemosus was considered.[18-20]

MATERIAL AND METHOD

Collection of Plant Material

The plant bark was collected from the market and aboveboard by a local Taxonomist. The plant material was washed consuming distilled water to remove the surface pollutants followed by air drying. The dried sample was powdered, stored in a sterile condition and used for further studies.

Extraction

35 grams of fine powder of plant material was extracted with 120 ml of an appropriate solvent (methanol/ethanol/acetone) in a round bottom flask with a magnetic stirrer for 6 hours, 12 hours and 24 hours. The bark extract was then centrifuged at 3000 rpm for 10 min each.

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Time	Methanol	Ethanol	Acetone
6 hours	1.148 gm	1.110 gm	0.819 gm
12 hours	1.424 gm	1.467 gm	0.962 gm
24 hours	1.875 gm	1.627 gm	1.418 gm

Preparation of combination
The combination was prepared by mixing API with diff. extracts. The ratio of API and plant extract was maintained 1:1

Antimicrobial Activities
The well-diffusion method, as advised by (Arodiya *et al*.) with some changes, was used to investigate the microbial activity of cinnamon different solvent extracts, antibiotics, and combinations (extracts + antibiotics). Muller Hinton agar medium was used. The spreading technique was used to inoculate bacteria and fungi. A 7 mm sterilized cup borer was used to create agar wells, which were then filled with 50 microliters of the tested soln. Petri dishes were incubated at 36 °C for 26 hours. The ZOI was used to express the inhibitory action of cinnamon, antibiotics, and combination in mm.

HPTLC
Prewashing of HPTLC plates
HPTLC plates (10 cm x 10 cm) were activated at 110 °C for 18 min using a TLC plate heater III after being washed with methanol.

Preparation of Standards
Methanol was used to prepare standard solutions of *Asparagus racemosus* methanol extract and antibiotics of conc. of 0.1 mg/ml. Equal amounts of each standard solution were combined to prepare a mixture of the standards.

Plate development and derivatization
The Linomat 5 semi-autosampler was used to apply the individual standard solutions and standard mixture as 2-lL bands in five tracks, 1 cm from the plate's base, with a bandwidth of 5 mm and spacing between bands of 2 mm. All tracks 1-5 on the plates received samples in the following order: antibiotic, methanol extract, anti + methanol extract, ethanol extract, and anti + ethanol extract. 10 mL of mobile phase were pre-saturated in HPTLC twin trough chambers (10 cm x 10 cm) for 10 minutes. Over a migration distance of 5 cm, the mobile phase was employed to resolve the adsorbed standard and standard mix after being dispersed equally across the twin trough chamber. The mobile phase was composed of n-butanol, ammonia, water, and DMSO (8:3:1:2). In a fume closet, plates were allowed to dry.

RESULT AND DISCUSSION
The extracts of the bark of *Asparagus racemosus* were compounded with API and exposed to antimicrobial property. Zone of inhibition is expressed in mm.

Antimicrobial Evaluation
The antibacterial activity of methanol, ethanol, and acetone extract of powder of *Asparagus racemosus* alone and with a combination of antibiotics was evaluated.
All three extracts of *A. racemosus* show effective antibacterial activity against tested bacterial species. Acetone/Ethanol/Methanol extract exhibited a zone of inhibition with the 1000 µg/ml concentration. All three extracts showed effective antibacterial activity against *P. aeruginosa* species. Acetone extract shows 05, 04, 08 and 10 mm ZOI against mentioned respective bacterial culture which is little lower than other ethanol and methanol extract. Ethanol has 08 mm, 07 mm, 11 mm and 13 mm zone of inhibition and methanol extract has 11 mm, 10 mm, 14 mm and 16 mm ZOI with 1 % w/v concentration **(Table 1)**.
Acetone extract has zone of inhibition 25 mm, 25 mm, 22 mm and 23 mm for 1% concentration while ethanol extract shows 34 mm, 33 mm, 30 mm and 32 mm ZOI for same concentration and methanol extract shows 21 mm, 28 mm, 25 mm and 26 mm zone of inhibition . Further all have higher resistance potential against *S. aereus* bacterial culture. Further as concentration reduce from 1% to 0.125% resistance potential decreases. This combination has good synergic effect and good resistance power against different mention bacteria. As amoxicillin inhibited *S. aureus* and *B. subtilis* growth very effectively, their combination with all three extracts also shows effective inhibitory activity against these two species amongst all tested bacteria **(Table 2)**.
Ciprofloxacin shows almost similar effects of antibacterial activity against all the used bacterial species. Combination of ciprofloxacin with acetone extract show 31 mm, 31 mm, 28 mm, 29 mm, with ethanol extract show 30 mm, 29 mm, 26 mm, 27 mm, and with methanol extract show 29 mm, 29 mm, 26 mm and 27 mm ZOI against *S. aureus, B. subtilis, P. aeruginosa,* and *E. coli* respectively at 1000 µg/ml concentration **(Table 3)**. So the combination of ciprofloxacin with all three extracts shows higher inhibition against *S. aureus* and *B. subtilis.*

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Ceftazidime exhibits the inhibitory effects in the same order as pure extracts against all strains of bacteria that have been used, and their combinations behave similarly. Combination have good synergic effect compare to pure Ceftidizime and also compare to pure extract. Combinations of Ceftazidime with acetone extract show 05 mm, 05 mm, 05 mm, and 05 mm with ethanol extract show 05 mm, 05 mm, 05 mm, and 05 mm and with methanol extract show 04 mm, 04 mm, 04 mm, 04 mm of ZOI against *S. aureus, B. subtilis, P. aeruginosa,* and *E. coli* respectively at 1.0% w/v concentration. Here one point noted combination of plant extract with Ceftazidime activity reduced against all stain **(Table 4)**.
Erythromycin and its combinations with all three extracts show higher activity against all the bacterial stain. Combination with acetone extract show 24 mm, 24 mm, 21 mm, and 22 mm; with ethanol extract show 27 mm, 26 mm, 23 mm, and 26 mm; and with methanol extract show 28 mm, 28 mm, 25 mm, and 26 mm of zone of inhibition against *S. aureus, B. subtilis, P. aeruginosa,* and *E. coli* respectively at 1.0%w/v concentration **(Table 5)**.
As the concentration of all three extracts decreased, the activity of their combinations with used antibiotics against all of the tested bacterial species also dropped.

Table 1: Antibacterial activity of pure extract

Bacteria	**Acetone Extract**				**Ethanol Extract**				**Methanol Extract**			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
S. aureus	05	05	04	03	08	07	05	03	11	09	06	03
B. subtilis	04	04	04	03	07	06	05	03	10	08	06	03
P. aeruginosa	08	08	07	06	11	10	08	06	14	12	09	06
E. coli	10	10	09	08	13	12	10	08	16	14	11	08

Table 2: Antibacterial activity of extract with Amoxicillin

Bacteria	**Acetone Extract + Amoxicillin**				**Ethanol Extract + Amoxicillin**				**Methanol Extract + Amoxicillin**			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
S. aureus	25	25	24	22	34	33	30	28	21	21	20	20
B. subtilis	25	25	24	22	33	32	30	28	28	28	25	25
P. aeruginosa	22	22	21	19	30	29	27	26	25	25	22	22
E. coli	23	23	22	20	32	31	28	27	26	26	23	23

Table 3: Antibacterial activity of extract with Ciprofloxacin

Bacteria	**Acetone Extract + Ciprofloxacin**				**Ethanol Extract + Ciprofloxacin**				**Methanol Extract + Ciprofloxacin**			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
S. aureus	31	30	29	28	30	30	30	29	29	29	28	28
B. subtilis	31	30	29	28	29	29	28	28	29	28	28	27
P. aeruginosa	28	27	26	25	27	27	26	25	27	26	26	25
E. coli	29	28	27	26	27	27	26	25	27	26	26	25

Table 4: Antibacterial activity of extract with Ceftazidime

Bacteria	**Acetone Extract + Ceftazidime**				**Ethanol Extract + Ceftazidime**				**Methanol Extract + Ceftazidime**			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
S. aureus	05	05	04	03	05	05	05	04	04	04	03	03
B. subtilis	05	05	04	03	05	05	05	05	04	04	03	03
P. aeruginosa	05	05	04	03	05	05	04	04	04	04	03	03
E. coli	05	05	04	03	05	05	04	04	04	04	03	03

Table 5: Antibacterial activity of extract with Erythromycin

Bacteria	**Acetone Extract + Erythromycin**				**Ethanol Extract + Erythromycin**				**Methanol Extract + Erythromycin**			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
S. aureus	24	24	23	22	27	27	26	26	28	28	26	25
B. subtilis	24	24	23	22	26	26	26	25	28	28	25	24
P. aeruginosa	21	21	20	19	23	23	22	21	25	25	24	23
E. coli	22	22	21	20	26	26	24	24	26	25	24	22

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Antifungal Evaluation

The antifungal activity of methanol, ethanol, and acetone extract of *A. racemosus* and their combination with two antifungal antibiotics was evaluated.

All three extracts of *A. racemosus* show effective antifungal activity against tested bacterial species. Acetone/Ethanol/Methanol extract exhibited a zone of inhibition with the 1000 µg/ml concentration. All three extracts showed lower antifungal activity against *A. niger* and *C. albicans* species. Acetone extract shows 05 mm and 05 mm ZOI. Ethanol has 07 mm and 08 mm zone of inhibition and methanol extract has 08 mm and 11 mm ZOI with 1000 µg/ml concentration **(Table 6)**.

Amphotericin-B showed minor activity against *A. niger* and *C. albicans*. All three extracts showed antifungal activity in the 03 mm to 05 mm range against *A. niger* and *C. albicans* **(Table 7)**. Amphotericin-B showed effective inhibition against both tested fungi, combining all extracts with amphotericin-B shows effective inhibition, but methanol, ethanol and acetone all three extract shows a more effective synergistic effect. Combination with acetone extract shows 05 mm and 05 mm zone of inhibition against both fungi and ethanol extract has 05 mm and 05 mm ZOI while methanol extract has 04 mm and 04 mm ZOI for 1.0 %w/v concentration.

Combination of plant extract with fluconazole show synergic effect and have resistance potential against both fungi. Combination has 15 mm and 25 mm ZOI for respective fungi for acetone extract. Same way for ethanol extract have 13 mm and 23 mm ZOI and for methanol extract has 14 mm and 24 mm ZOI for respected mentioned fungi for 1%w/v concentration. These results revealed that combinations of acetone, ethanol and ethanol, extract with fluconazole are more effective against both the tested fungi **(Table 8)**.

Table 6: Antifungal activity of pure extract

Fungus	**Acetone Extract**				**Ethanol Extract**				**Methanol Extract**			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
A. niger	05	05	04	03	07	05	05	03	08	06	06	03
C. albicans	05	05	04	03	08	07	05	03	11	09	06	03

Table 7: Antifungal activity of extract with Amphotericin-B

Fungus	**Acetone Extract + Amphotericin-B**				**Ethanol Extract + Amphotericin-B**				**Methanol Extract + Amphotericin-B**			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
A. niger	05	05	04	03	05	05	05	05	04	04	03	03
C. albicans	05	05	04	03	05	05	05	05	04	04	03	03

Table 8: Antifungal activity of extract with Fluconazole

Fungus	**Acetone Extract + Fluconazole**				**Ethanol Extract + Fluconazole**				**Methanol Extract + Fluconazole**			
Conc. in µg/ml	1000	500	250	125	1000	500	250	125	1000	500	250	125
A. niger	15	15	12	11	13	13	13	12	14	14	13	12
C. albicans	25	25	22	21	23	23	23	22	24	24	23	22

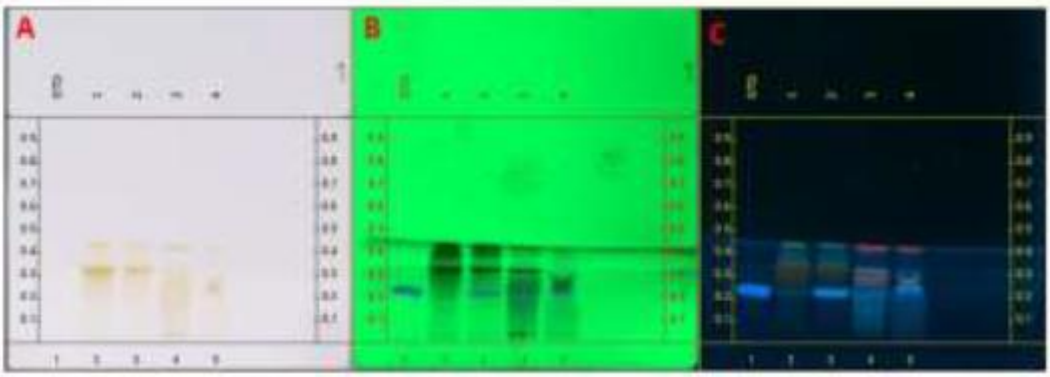

Figure 1: HPTLC of Amoxicillin, extracts and combination at (A) Visible, (B) UV 254 nm, (C) UV 366 nm.

In Figure 1 (A/B/C) represent the same HPTLC experiments result in diff. lights. The first band of Amoxicillin also present at the same Rf value in the third and fifth band which is the combination of Amoxicillin and methanol extract &

Amoxicillin and ethanol extract respectively. No any new band is visible and no old band has Disappeared which confirms no any new compound formed by combining antibiotics and extract and so why no further study require for toxicity.

CONCLUSIONS

Effective antimicrobial activity against microorganisms has been demonstrated by plant extract. It can be used to enhance the antimicrobial activity of antibiotics and so as well we can reduce side effect of antibiotics by decreasing their concentrations.

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Conflict of Interest

The authors confirm that this article's content has no conflict of interest.

REFERENCES

1. Goyal, R. K., Singh, J., & Lal, H. (2003). Asparagus racemosus-an update. *Indian journal of medical sciences*, *57*(9), 408-414.
2. Alok, S., Jain, S. K., Verma, A., Kumar, M., Mahor, A., & Sabharwal, M. (2013). Plant profile, phytochemistry and pharmacology of Asparagus racemosus (Shatavari): A review. *Asian Pacific journal of tropical disease*, *3*(3), 242-251.
3. Hasan, N., Ahmad, N., Zohrameena, S., Khalid, M., & Akhtar, J. (2016). Asparagus racemosus: for medicinal uses & pharmacological actions. *International Journal of Advanced Research*, *4*(3), 259-267.
4. Potduang, B., Meeploy, M., Giwanon, R., Benmart, Y., Kaewduang, M., & Supatanakul, W. (2008). Biological activities of Asparagus racemosus. *African Journal of Traditional, Complementary and Alternative Medicines*, *5*(3), 230-237.
5. Sachan, A. K., Das, D. R., Dohare, S. L., & Shuaib, M. (2012). Asparagus racemosus (Shatavari): an overview. *Int J Pharm Chem Sci*, *1*(3), 588-592.
6. Mandal, S. C., Nandy, A., Pal, M., & Saha, B. P. (2000). Evaluation of antibacterial activity of Asparagus racemosus Willd. root. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *14*(2), 118-119.
7. Battu, G. R., & Kumar, B. M. (2010). Phytochemical and antimicrobial activity of leaf extract of Asparagus racemosus Willd. *Pharmacognosy Journal*, *2*(12), 456-463.
8. Sairam, K., Priyambada, S., Aryya, N. C., & Goel, R. K. (2003). Gastroduodenal ulcer protective activity of Asparagus racemosus: an experimental, biochemical and histological study. *Journal of ethnopharmacology*, *86*(1), 1-10.
9. Hayes, P. Y., Jahidin, A. H., Lehmann, R., Penman, K., Kitching, W., & De Voss, J. J. (2008). Steroidal saponins from the roots of Asparagus racemosus. *Phytochemistry*, *69*(3), 796-804.
10. Mishra, J. N., & Verma, N. K. (2017). Asparagus racemosus: Chemical constituents and pharmacological activities—A review. *Eur. J. Biomed. Pharm. Sci*, *4*, 207-213.
11. Singh, G. K., Garabadu, D., Muruganandam, A. V., Joshi, V. K., & Krishnamurthy, S. (2009). Antidepressant activity of Asparagus racemosus in rodent models. *Pharmacology Biochemistry and Behavior*, *91*(3), 283-290.
12. Kumar, S., Mehla, R. K., & Dang, A. K. (2008). Use of shatavari (Asparagus racemosus) as a galactopoietic and therapeutic herb–a review. *Agricultural Reviews*, *29*(2), 132-138.
13. Venkatesan, N., Thiyagarajan, V., Narayanan, S., Arul, A., Raja, S., Kumar, S. V., ... & Perianayagam, J. B. (2005). Anti-diarrhoeal potential of Asparagus racemosus wild root extracts in laboratory animals. *J Pharm Pharm Sci*, *8*(1), 39-46.
14. Choudhary, D., & Sharma, D. (2014). A phytopharmacological review on Asparagus racemosus. *Int J Sci Res*, *3*(7), 742-746.
15. Sharma, M., Sharma, A., & Kumar, A. (2012). Vital medicine Asparagus racemosus willd. *Current Trends in Biotechnology and Pharmacy*, *6*(2), 210-221.
16. Velavan, S., Nagulendran, K. R., Mahesh, R., & Begum, V. H. (2007). Phcog Rev.: plant review the chemistry, pharmacological and therapeutic applications of Asparagus racemosus-a review. *Pharmacognosy Reviews*, *1*(2), 350-360.
17. Sharma, A., Chadha, N. K., Das, S. K., Sen, A., Roy, S., Chanu, T. I., ... & Prakash, C. (2018). Asparagus racemosus aqueous root extract induced effects on cellular immune reaction of Labeo rohita (Hamilton). *Indian Journal of Animal Sciences*, *88*(2), 251-258.
18. Kumar, A. S. H. W. A. N. I., & Vijay, N. E. E. T. U. (2009). In vitro plantlet regeneration in Asparagus racemosus through shoot bud differentiation on nodal segments.

19. Vijay, N., Kumar, A., & Bhoite, A. (2009). Influence of nitrogen, phosphorus and potassium fertilizer on biochemical contents of Asparagus racemosus (Willd.) root tubers.
20. Sharma, M., Sharma, A., & Kumar, A. (2011). Ethnopharmacological importance of Asparagus racemosus: A review. *J Pharm Biomed Sci*, *6*(06).