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Study of certain medicinal plants of Sidhi district and their antimicrobial activity against plant and human pathogens

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Abstract
The methanol leaf extracts of *Acacia nilotica, Sida cordifolia, Tinospora cordifolia, Withania somnifer* and *Ziziphus mauritiana* are ethnomedicinally important showed significant antibacterial and activity against *Bacillus subtilis, Escherichia coli*, *Pseudomonas fluorescens, Staphylococcus aureus* and *Xanthomonas axonopodis* pv. *malvacearum* and antifungal activity against *Aspergillus flavus, Dreschlera turcica* and *Fusarium verticillioides* when compare to root/ bark extracts. *A. nilotica* and *S. cordifolia* leaf extract showed highest antibacterial activity against *B. subtilis*. *and Z. mauritiana leaf* extract showed significant activity against *X. a.* pv. *malvacearum.* Root and leaf extract of *S. cordifolia* recorded significant activity against all the test bacteria. *A. nilotica* bark and leaf extract showed significant antifungal activity against *A. flavus, Ziziphus mauritiana and Tinospora cordifolia recorded* significant antifungal activity against *D. turcica* The methanol extract of *Sida cordifolia* exhibited significant antifungal activity against *F. verticillioides*.

Keywords: *ethno-m*edicinal plants, antibacterial, antifungal

1. Introduction
Sidhi district is located on the Northeastern Boundary of the state between 22,475 and 24.4210 North Latitude and 81:1840 and 82.4830 East longitude. The district has Singrauli district in the north-east, Koriya district of Chhattisgarh on the east, and Rewa district on the west. According to the 2011 census, Sidhi District has a population of 1,126,515, roughly equal to the nation of Cyprus or the US state of Rhode Island. This gives it a ranking of 411th in India (out of a total of 640). The district has a population density of 232 inhabitants per square kilometre (600/sq mi). Its population growth rate over the decade 2001-2011 was 23.66%. Sidhi has a sex ratio of 952 females for every 1000 males, and a literacy rate of 66.09%. In Sidhi district Gond, Kol, Baiga tribes are mostly found.

The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. Thus, any phytochemical investigation of a given plant will reveal only a very narrow spectrum of its constituents. Historically pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. Random screening as tool in discovering new biologically active molecules has been most productive in the area of antibiotics (Gerhartz *et al.* 1985) [1]. Even now, contrary to common belief, drugs from higher plants continue to occupy an important niche in modern medicine. On a global basis, at least 130 drugs, all single chemical entities extracted from higher plants, or modified further synthetically, are currently in use, though some of them are now being made synthetically for economic reasons (Newman *et al.* 2000) [2].

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava *et al.* 1996) [3]. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many

herbal industries (Uniyal *et al.* 2006) [4]. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated (Balandrin *et al.* 1985) [5]. Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial and antifungal agents, a systematic investigation was undertaken to screen the local flora for antibacterial and antifungal activity from *Acacia nilotica, Sida cordifolia*, *Tinospora cordifolia, Ziziphus mauritiana and Withania somnifera.* The *Acacia nilotica* (L.) Willd. belongs to the family Mimosaceae. Decoction of bark is used as gargle and of pods in urino-genital diseases. *Sida cordifolia* L. (Malvaceae) root is used in pain, nervous disorders, coryza, cardiac diseases. The stem of *Tinospora cordifolia* (Wild.) Hk.f. & Th. which belongs to the family Menispermaceae is used as an ingredient for Ayurvedic preparations used in general debility, dyspepsia, fevers and urinary diseases. Root is a powerful emetic and used for visceral obstruction; its watery extracts is used in leprosy. *Withania somnifera* (L.) Dunal which belong to the family Solanaceae is used in hiccup, cough, dropsy, rheumatism and gynaecological disorders and as a sedative in senile debility. It is also useful in inflammatory conditions, ulcers and scabies as external application. Leaves used as a febrifuge and applied to lesions painful swellings and sore eyes. *Ziziphus mauritiana* Lam. Belongs to the family Rhamnaceae. The fruits used anodyne and tonic. They form one of the ingredients of Joshanda, a medicine used in chest troubles. Kernels sedative, used as soporific and to stop vomiting; also employed as an antidote to aconite-poisoning and in abdominal pains. Seeds are given in diarrhea. Leaves with catechu (*Areca catechu*) used as an astringent and considered diaphoretic (Yoganarasimhan, 1996) [6].

2. Material and Methods

Ethnomedicinal uses

The present work is based on the results of two years. During the course of study a large number of rural persons including tribal men were interviewed together the ethnobotanical information through specially prepared questionnaire. Selected villages of three tahasils were surveyed and ethnobotanical information and plant specimens were collected

Antimicrobial study

Collection of Plant Material: Fresh leaves bark and root of five different plants viz., *Acacia nilotica* (L.) Willd., *Sida cordifolia* L., *Tinospora cordifolia* (Wild.) Hk.f. & Th., *Withania somnifera* (L.) Dunal and *Ziziphus mauritian* Lam. free from disease were collected from various forest pockets of Sidhi district. The leaves with sterile distilled water, leaf, bark and root material was then air-dried on sterile blotter under shade.

Solvent Extraction: Thoroughly washed dried leaves of five plants of *Acacia nilotica, Sida cordifolia Tinospora cordifolia, Withania somnifera* and *Ziziphus mauritian*, bark extract of *Acacia nilotica Tinospora cordifolia* and *Ziziphus mauritian* and root extract of *Sida cordifolia* and *Withania somnifera* of plant material were dried in shade for five days and then powdered with the help of Waring blender. 25 g of shade-dried powder was filled in the thimble and extracted successively with methanol solvent in Soxhlet extractor for 48h. The solvent extracts were concentrated under reduced pressure and preserved at 5°C in airtight bottle until further use.

Growth and Maintenance of Test Microorganism for Antimicrobial Studies: Bacterial cultures of *Bacillus subtilis* (*B. subtilis*), *Escherichia coli* (*E. coli*), *Pseudomonas fluorescens* (*P. fluorescens*), *Staphylococcus aureus* (*S. aureus*) and *Xanthomonas axonopodis* pv. *malvacearum (X. axonopodis* pv. *malvacearum)* and fungal cultures of *Aspergillus flavus* (*A. flavus*), *Dreschlera turcica* (*D. turcica*) and *Fusarium verticillioides* (*F. verticillioides*), were obtained from the culture collection centre, Department of microbiology and Biotechnology, Awadhesh Pratap Singh University, Rewa (M.P.), India, were used for antimicrobial test organisms. The bacteria were maintained on nutrient broth (NB) at 37°C and fungus were maintained on Potato dextrose agar (PDA) at 28°C.

Preparation of Inoculum: The gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli, Pseudomonas fluorescens* and *Xanthomonas axonopodis pv. malvacearum*) were pre-cultured in nutrient broth overnight in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{610} nm). The fungal inoculum (*F. verticillioides, D. turcica* and *A. flavus*) was prepared from 5 to 10 day ole culture grown on Potato dextrose agar medium. The Petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was adjusted with spectrophotometer (A_{595} nm) to obtain a final concentration of approximately 10^5 spores/ml.

Anti-bacterial Activity: The methanol leaf extracts of *Acacia nilotica, Sida cordifolia Tinospora cordifolia, Withania somnifera* and *Ziziphus mauritian*, bark extract of *Acacia nilotica Tinospora cordifolia* and *Ziziphus mauritian* and root extract of *Sida cordifolia* and *Withania somnifera* were tested by the disc diffusion method (Anonymous, 1996) [7]. Different concentration of the extracts (100 µg ml) was prepared by reconstituting with methanol. The test microorganisms were seeded into respective medium by spread plate method 10 µl (10^6 cells/ml) with the 24h cultures of bacteria growth in nutrient broth. After solidification the filter paper discs (5 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. *B. subtilis, E. coli*, *P. fluorescens S. aureus* and *X. a*. pv. *malvacearum* were used for antibacterial test. Streptomycin sulphate (10 µg ml) used as positive control and methanol solvent (100 µg ml 1) used as negative control The antibacterial assay plates were incubated at 37°C for 24h. The diameters of the inhibition zones were measured in mm.

Antifungal Activity: The antifungal activity was tested by disc diffusion method (Taylor *et al.* 1995)[8]. The potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. The filter paper discs (5 mm in diameter) impregnated with 100 µg ml 1 concentrations of the extracts were placed on test organism-seeded plates. Methanol was used to dissolve the extract and was completely evaporated before application on test organism-seeded plates. Blank disc impregnated with

solvent methanol followed by drying off was used as negative control and Nystatin (10 µg disc^{-1}) used as positive control. The activity was determined after 72 h of incubation at 28°C. The diameters of the inhibition zones were measured in mm.

3. Results

Results obtained in the present study relieved that the tested five medicinal plants extracts posses potential antibacterial activity against *B. subtilis, E. coli, X. axonopodis pv. malvacearum, S. aureus, P. fluorescens,* (Table 1) and antifungal activity against *F. verticillioides, D. turcica* and *A. flavus* (Table 2). When tested by the disc diffusion method, the methanol leaf extracts of *Acacia nilotica* showed significant activity against *E. coli, S. aureus* and *X. a.* pv. *malvacearum* around 15 mm. The highest antibacterial activity of 20 mm in *B. subtilis* and least activity recorded in *E. coli* measured 14 mm. Bark extract of *Acacia nilotica* exhibit highest activity against *B. subtilis* and *S. aureus* (15 mm) and lowest in *P. fluorescens*. *Sida cordifolia* leaf extract posses maximum activity against *B. subtilis* and *S. aureus* (18 mm) and the 16 mm similar zone of inhibition observed in *E. coli*, *P. fluorescens* and *X. a.* pv. *malvacearum*. Root extract of this plant showed highest inhibitory activity against *B. subtilis* and *S. aureus* and least activity observed in *E. coli*. *Tinospora cordifolia* leaf extracts showed almost similar zone of inhibition against all the tested bacteria except *X. a.* pv. *malvacearum*, which showed highest activity (17 mm).

Table 1: Antibacterial activity of some medicinal plant methanol extracts (100 µg ml^{-1}) and antibiotic (10 µg ml^{-1}) against bacterial species tested by disc diffusion assay

S. No.	Bacterial sp.	Zone of inhibition (mm)										Steptomycin sulphate
		Acacia nilotica		*Sida cordifolia*		*Tinospora cordifolia*		*Withania somnifera*		*Ziziphus mauritiana*		
		Bark	Leaves	Root	Leaves	Bark	Leaves	Root	Leaves	Bark	Leaves	
1.	*Bacillus subtilis*	15±0.67	20±1.21	16±0.32	18±0.56	13±0.78	15±0.50	14±0.56	15±0.32	11±0.33	15±0.34	16±0.34
2.	*Escherichia coli*	14±0.31	15±0.87	12±0.33	15±0.87	10±0.78	14±0.04	15±0.01	16±0.55	13±0.56	18±0.57	18±0.34
3.	*Pseudomonas fluorescense*	11±0.56	14±0.32	14±1.23	16±1.14	12±0.88	14±0.17	14±0.32	15±0.34	11±0.57	15±0.33	14±0.34
4.	*Staphylococcus aureus*	15±0.67	15±0.87	16±0.33	18±1.20	12±0.88	15±0.50	14±0.67	15±0.34	14±0.67	14±0.55	15±0.32
5.	*Xanthonomonas axonopodies*	13±0.56	15±0.87	13±0.87	16±0.34	14±0.24	17±0.52	14±0.58	16±0.67	13±0.32	18±0.87	16±0.32

Values are mean inhibition zone (mm) ± S.D of three replicates

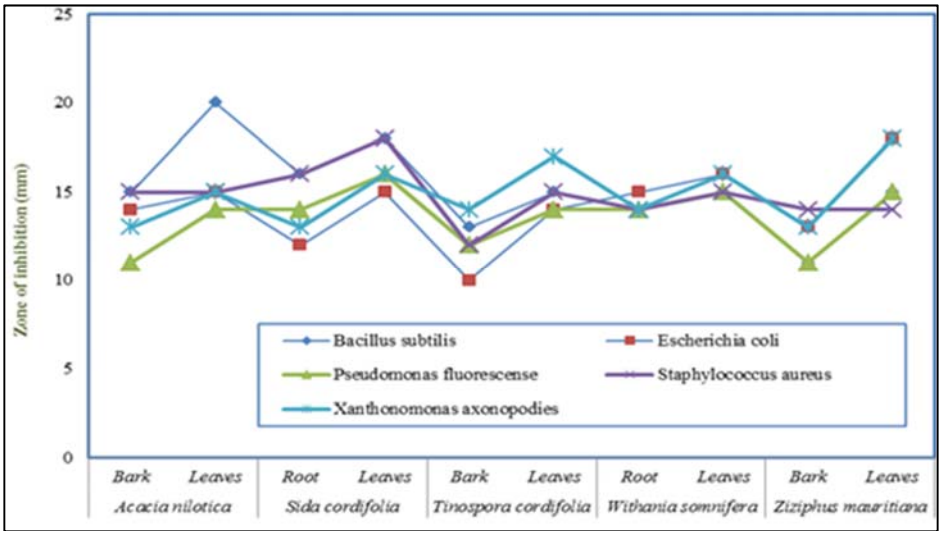

Fig. 1: Antibacterial activity of some medicinal plant methanol extracts (100 µg ml^{-1}) and antibiotic (10 µg ml^{-1}) against bacterial species tested by disc diffusion assay

Table 2: Antifungal activity of some medicinal plant methanol extracts (100 µg ml^{-1}) and fungicide (10 µg ml^{-1}) against fungal species tested by disc diffusion assay

S. No.	Fungal sp.	Zone of inhibition (mm)										Nystatin
		Acacia nilotica		*Sida cordifolia*		*Tinospora cordifolia*		*Withania somnifera*		*Ziziphus mauritiana*		
		Bark	Leaves	Root	Leaves	Bark	Leaves	Root	Leaves	Bark	Leaves	
1.	*Aspergillus flavus*	12±0.56	12±0.32	8±0.34	8±0.56	9±0.33	9±0.34	7±0.34	7±0.01	10±0.67	11±0.87	19±0.34
2.	*Dreschlera turcica*	8±0.34	10±0.35	8±0.32	9±0.34	13±0.32	14±0.55	13±0.31	14±0.59	12±0.60	11±0.32	22±0.58
3.	*Fusarium verticillioides*	8±0.32	9±0.01	10±0.67	12±0.31	11±0.30	10±0.34	11±0.33	10±0.32	8±0.30	9±0.34	16±0.35

Values are mean inhibition zone (mm) ± S.D of three replicates

Bark extract of *Tinospora cordifolia* showed varied in the zone of inhibition from 10-14 mm against all the tested bacteria. Root and leaf extract of *Withania somifera* showed almost similar antibacterial activity against all the tested bacteria. Leaf extract of *Ziziphus mauritiana* showed highest activity against *E. coli* and *X. a.* pv. *malvacearum* (18 mm) lowest activity were observed in *S. aureus*, *P. fluorescens* and *B. subtilis* around 15 mm zone of inhibition. Bark extract of this plant showed significant activity against *S. aureus* followed by *E. coli*, *X. a*. pv. *malvacearum* and the minimum activity were observed in *B. subtilis* and *P. fluorescens*. Among the five plants viz., *Acacia nilotica, Sida cordifolia Tinospora cordifolia, Withania somnifera* and *Ziziphus mauritian,* leaf and bark extract showed significant antibacterial activity against the test pathogens. Leaf extract showed significant activity when compared with the bark/root extract of all the test plant extract. Bark extract of all the five plant extracts was almost similar or higher activity when compared with the streptomycin sulphate.

Antifungal activity of five plant leaf extract showed significant activity when compared with the bark/root extract. *Acacia nilotica* bark and leaf extract showed antifungal activity against *Aspergillus flavus* (12 mm) followed by leaf extract of *Zizphus mauritian* (11 mm). *Tinospora cordifolia* and *Withania somnifera* bark extract recorded better activity against *Dreschlera turcica* followed by leaf extract of *Tinospora cordifolia* and *Withania somnifera*. *Fusarium verticillioides* recorded susceptibility for all the five plant leaf and bark / root extract. All the five plants viz., *Acacia nilotica, Sida cordifolia Tinospora cordifolia, Withania somnifera* and *Ziziphus mauritian* showed less antifungal activity when compared with Nystatin.

4. Discussion

The rural and tribal men are well versed with the symptoms of various types of diseases and with their herbal remedies because they have carried on practice traditionally by verbal instruction. Moreover it has been observed that although modern medical facilities are approachable at places, still they prefer to use herbal drugs owing to their confidence and belief in such treatment. In view of the fact that the medicinal uses of plants have been confidently claimed by the rural and tribal people, detailed pharmacological and clinical studies are needed to ascertain their role in modern medicines.

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay (Tona *et al.* 1998) [9]. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants (Govindarajan *et al.* 2006, Behera *et al.* 2005, Palombo and Semple 2001 and Stepanovic *et al.* 2003) [10-13]. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings. However, not many reports are available on the exploitation of antifungal or antibacterial property of plants for developing commercial formulations for applications in crop protection.

In the present study, the methanol leaf, root/bark extracts of *Acacia nilotica, Sida cordifolia Tinospora cordifolia, Withania somnifera* and *Ziziphus Mauritian* showed the activity against *B. subtilis, E. coli, P. fluorescens, S. aureus, X. axonopodis* pv. *malvacearum, A. flavus*, *D. turcica* and *F. verticillioides* and Plant based products have been effectively proven for their utilization as source for antimicrobial compounds. For instance, methanol extracts of *A. ferox* and *W. somnifera* exhibited inhibitory activity against all the strains of *N. gonorrhoea* while, only the methanol extract of *W. somnifera* was effective against *C. albicans* (Kambizi and Afolayan, 2008) [14]. The antibacterial activity of aqueous, different solvent extracts and isolated constituents of leaves of *Acacia nilotica* were evaluated by the cup diffusion method against three phytopathogenic *Xanthomonas* pathovars viz., *Xanthomonas axonopodis* pv. *phasedi*, *X. axonopodis pv. malvacearum* and *X. axonopodis* pv. *vesicatoria.* Methanol extract was subsequently fractioned and monitored by bioassay leading to the isolation of active fraction by further phytochemical analysis (Raghavendra *et al.* 2006) [15]. Futher, monomeric glycoprotein namely, WSG (*Withania somnifera* glycoprotein) isolated from *W. somnifera* root tubers revealed (protease inhibitor) antimicrobial activity against few bacterial and phytopathogenic fungi (Girish *et al*. 2006) [16]. WSG also provided a fungi stastic effect by inhibiting spore germination and hyphal growth in the tested fungi.

On the contrary, (Afolayan, 2002) [17] observed that water, methanol and acetone extracts did not have activity on *S. marcescens*, a gram-negative bacterium. Apart from antimicrobial activities, these plant extracts are also exploited for therapeutic purpose to cure several disorders. Methanol extract of the root of *Z. mauritiana* was found to inhibit the severity of diarrhoea induced by castor oil. It is speculated that the extract was able to inhibit electrolyte permeability in the intestine due to castor oil and or through the inhibition of prostaglandins release (Adzu *et al*. 2003) [18]. The results of present investigation clearly indicate that the antibacterial and antifungal activity vary with the species of the plants and plant material used. Thus, the study ascertains the value of plants used in ayurveda, which could be of considerable interest to the development of new drugs.

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6. References

1. Gerhartz W, Yamamota YS, Campbell FT, Pfefferkorn R, Rounsaville JF. Ullmann's Encyclopedia of Industrial, 1985.
2. Newman DJ, Cragg GM, Snader KM. The influence of natural products upon drug discovery. Nat. Prod. Res. 2000; 17:215-234.
3. Srivastava J, Lambert J, Vietmeyer N. Medicinal plants: An expanding role in development. World Bank Technical Paper. No. 320, 1996.
4. Uniyal SK, Singh KN, Jamwal P, Lal B. Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalayan. J. Ethnobiol. Ethnomed. 2006; 2:1-14.
5. Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH. Natural plant chemicals: Sources of Industrial and Medicinal materials. Science, 1985; 228:1154-1160.

6. Yoganarasimhan N. Medicinal plants of India Vol. I, Interline Publishing Pvt., Ltd., Bangalore, India, 1996.
7. Anonymous. Pharmacopiea of India (The Indian Pharmacopiea), 3rd Edn., Govt. of India, New Delhi, Ministry of Health and Family Welfare, 1996.
8. Taylor RSL, Manandhar NP, Hudson JB, Towers GHN. Screening of selected medicinal plants of Nepal for antimicrobial activities. J. Ethnopharmacol. 1995; 546:153-159.
9. Tona L, Kambu K, Ngimbi N, Cimanga K, Vlietinck AJ. Antiamoebic and phytochemical screening of some Congolese medicinal plants. J. Ethnopharmacol. 1998; 61:57-65.
10. Govindarajan R, Vijayakumar M, Singh M, Rao CHV, Shirwaikar A, Rawat AKS *et al*. Antiulcer and antimicrobial activity of Anogeissus latifolia. J. Ethnopharmacol. 2006; 106:57-61.
11. Behera SK, Misra MK. Indigenous phytotherapy for genito-urinary diseases used by the Kandha tribe of Orissa, India. J. Ethnopharmacol. 2005; 102:319-325.
12. Palombo EA, Semple SJ. Antibacterial activity of traditional medicinal plants. J. Ethnopharmacol., 2001; 77:151-157.
13. Stepanovic S, Antic N, Dakic I, Svabic-vlahovic M. *In vitro* antimicrobial activity of propilis and antimicrobial drugs. Microbiol. Res. 2003; 158:353-357.
14. Kambizi L, Afolayan AJ. Extracts from *Aloe ferox* and *Withania somnifera* inhibit *Candida albicans* and Neisseria gonorrhoea. African J. Biotechnol., 2008; 7: 12-15.
15. Raghavendra MP, Satish S, Raveesha KA. Phytochemical analysis and antibacterial activity of *Oxalis corniculata*: A known medicinal plant. My. Science. 2006; 1:72-78.
16. Girish KS, Machiah KD, Ushanandini S, Kumar H, Nagaraju KS, Govindappa M *et al*. Antimicrobial properties of a non-toxic glycoprotein (WSG) from *Withania somnifera* (Ashwagandha). J. Basic Microbiol. 2006; 46:365-374.
17. Afolayan AJ, Grierson DS, Kambizi L, Madamombe I, Masika PJ. *In vitro* antifungal activity of some South African medicinal plants. S. Afri. J. Bot. 2002; 68:72-76.
18. Adzu BS, Amos S, Amizan MB, Gamaniel KS. Evaluation of the antidiarrheal effects of Z. Spina-christi Sterm bark in rats. Acta Tropica, 2003; 6:1-5.