A PUBLICATION OF MOTHERHOOD UNIVERSITY, ROORKEE

(Recognized by the UGC with the right to award degrees u/s 22(1) of the UGC act 1956 and established under Uttarakhand Government Act No. 05 of 2015)

Motherhood International Journal of Multidisciplinary Research & Development

A Peer Reviewed Refereed International Research Journal

Volume I, Issue II, November 2016, pp. 30-36

ONLINE ISSN-2456-2831

In Vitro Biological Activity of Crude Extracts of Fruit (Seed material) Stereospermum suaveolens (D.C.) , Bignoniaceae

Dr. Yatendra Verma[1], Dr. S.B Sharm[2], Dr. A.K Molik[3], Aparna Verma[4]

[2]Dean, Faculty of Sciences, Motherhood University, Roorkee

[3]Ayurvedic Scientist, Roorkee

Uttarakhand, India

Abstract

The present study was designed to obtain preliminary information on In Vitro Biological activity and efficacy of four crude extracts of air shaded dried fruits (seed material) ***Stereospermum suaveolens, Bignoniaceae*** *selected on the basis of their medicinal uses and determined by the NCCLS method for fungus using RPMI-1640media buffered with MOPS (3-{N-morpholino} propanesulfonic acid), (sigma chemical co.) and Mueller Hinton broth for bacteria.*

Results representing the sensitivity of the bacterial and fungal tests correlated well the traditional uses of this plant, thus indicating the presence of potential antimicrobial agents in this medicinal plant. The microorganisms Streptococcus faecalis, Klebsiella pneumoniae, E-coli, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans, Cryptocloccus neoformans, Sporothrix schenckii, Trichophyton mentagrophytes, Aspergillus fumigatus, Candida parapsilosis (ATCC-22019) were found to be highly sensitive to analyzed fruits extracts. The antimicrobial activity of four crude extracts of air shaded dried fruits (seed material) can explain the potential use of ***Stereospermum suaveolens*** *in the eliminate or reduce Nausea, Vomiting, Headache, Migraine, Giddiness, Hemicrania and other associated symptom which focus for isolation of individual active principles and the subsequent pharmacological evaluation of the pure compound.*

Keywords: Vitro biological activity, Stereospermum suaveolens, Bignoniaceae.

Introduction

Very little is known about traditional herbal remedies in spite of the interesting therapeutic values and remarkable physiological effects that they have demonstrated in areas where disease of microbial origin are prevalent and contribute tremendously to the poor healthy conditions of the majority of population. There is no systematic scrutiny of therapeutic claims, and the biological activities of must of those healing plants have rarely been investigated studies involving traditional medicines frequently contribute new ideas. Numerous encouraging leads have come up with the convergence of empirical uses of various

species in various parts of the globe showing potential antimicrobial property. Antimicrobial activity of plants can be detected by observing the growth response of various microorganisms to those plant tissues or extracts which are placed in contact with them[1, 2].

In the past few years, a good deal of interest has been directed towards the scientific investigation of Indian medicinal plants that herbalists use in their daily practices. Several investigated plants proved to contain biologically active compounds which could be used for many human and animal's disorders. Such plants therefore deserve proper biological evaluation in order to identify their active principles as well as other useful bioactive constituents[1, 2]. Usually for the screening of the antibacterial activity of different plants extracts of varying polarity like petroleum ether, acetone, chloroform and ethanol, extracts of plant parts are screened for their activity by the antimicrobial screening method. After preliminary screening, the effective extracts are further investigated for their activity at different concentrations and the optimum activity of the specific extracts is measured[2]. *Aswal etal*, have described the results of their scientific endeavors in which the alcoholic extracts of 266 botanically identified plants materials from 222 plant spices were tested for various biological activities including chemotherapeutic and pharmacological[3].

In continuation of our study on biological activity of crude extracts of Fruit parts of ***Stereospermum suaveolens (DC)*** were carried out. These Extracts were tested for in *vitro* antibacterial and antifungal activities. The microorganisms *Candida albicans,. Cryptocloccus neoformans, Sporothrix schenckii, Trichophyton mentagrophyts, Aspergillus fumigatus,* and *Candida parapsilosis* were found to be highly sensitive to analyzed fruits extracts.

Materials and Methods

Plant Materials:

Air shaded dried at 35^0 C for one week fruits (seed material) ***Stereospermum suaveolens, Bignoniaceae*** were collected from Gurukul Kangri University Campus and forests of Shivalik range nearby Haridwar (U.A.), during April 2003. They were identified in the *Taxonomy* division of the NBRI, Lucknow where voucher specimens have been preserved.

Microorganisms:

Some of the microorganisms used in this investigation were laboratory strains provided by **Dr.A.K.Goel** *(Senior Assistant Director*) of the department of Fermentation Technology ***(Laboratory of Microbiology, CDRI Lucknow).*** They were *Streptococcus faecalis, Klebsiella pneumoniae,E-coli,Pseudomonas* aeruginosa , *Staphylococcus aureus, Candida albicans, Cryptocloccus neoformans, Sporothrix schenckii, Trichophyton mentagrophytes, Aspergillus fumigatus, Candida parapsilosis* **(ATCC-22019)**.The activity of compounds was determined by the NCCLS method for fungus using RPMI-1640media buffered with MOPS (3-{N-morpholino}propanesulfonic acid), (sigma chemical co.) and Mueller Hinton broth for bacteria.

Extraction:

Air shaded dried at 35^0 C for one week fruits (seed material, 565.5g) were exhaustively extracted with petroleum ether, chloroform , ethyl alcohol, and DDW (300-1000ml) at room temperature for 2-3

week using a *Soxhler extractor* . The solvent was removed at a low temperature under reduced pressure to yield thick syrup. Afforded organic solutions were dried over anhydrous sodium sulfate, filtered and concentrated to give organic extracts, Samples from petroleum ether, chloroform , ethyl alcohol, and DDW as well as lyophilized aqueous fractions were used to test for the antibacterial and antifungal activities.

Screening Methods for Antimicrobial Activity

Various methods have been used for the evaluation of *In Vitro* Biological activity. The commonly used methods are:

SERIAL DILUTION METHOD

The fungal and the bacterial strains were grown on Sabroaud dextrose agar and nutrient agar media respectively. After the incubation fungal and bacterial growth were suspended in normal saline and maintained at 1.0-5.0X10^3 cfµ/ml. The activity of compounds was determined by the **NCCLS** method for fungus using **RPLMI- 1640** media buffered with **MOPS(3-[N-morpholino] propanesultonic acid)** (Sigma Chemical Co.) and **Mueller Hinton broth** for bacteria. The 96-well tissue culture plates were used for two fold serial dilution. The proper growth control, drug control, drug control and the blank were adjusted onto the plate.

Compounds were dissolved in DMSO at a concentration of 10mg/ml and 20ul of this was added to **96-well tissue** culture having 180µl **RPMI- 1640** so the maximum concentration of the compound became 500 µg/ml. From here the solution was serially diluted resulting into the space half of the concentration of test compounds and then inoculums was added and kept for incubation. Micro-titer plates were incubated at 35^0C in a moist, dark chamber and MICs were recorded spectrophotometrically by micro titer plate reader (Molecular Devices). [4-5]

In this method [(6)], graded concentration of the test substance in a nutrient medium are inoculated with the organism and then incubated. The minimum concentration preventing the detectable growth of the bacteria (MIC) is taken as a measure of becteriostatics activity. Some idea of the effectiveness of a chemotherapeutic agent against a microorganism can be obtained from the minimal inhibitory concentration (MIC). The MIC is the lowest concentration of a drug that prevents growth of a particular microorganism. The minimal lethal concentration is the lowest drug concentration that kills the microorganism.

A coda drug kills microorganism at level only two to four-times the MIC Whereas, a static agent, kills at much higher concentrations Less the MIC for a compound more the antibacterial activity of that compound.

Results and Discussion

In Vitro Biological activity and efficacy of four crude extracts of air shaded dried fruits (seed material) ***Stereospermum suaveolens, Bignoniaceae*** in different solvents extracts which were tested for antibacterial and antifungal activities. Results from the bioassay are summarized in Table 1 and graphical represented by Fig. No. 1 and 2 respectively.

Examination of data in Table 1 clearly shows that most of the fruit extracts in Petroleum ether , chloroform and ethyl alcohol exhibited distinct zones of inhibition and thereby indicated the antimicrobial property of collected fruit materials. It was also observed (Table 1 and Fig. no.1) that active extracts showed a significant qualitative antibacterial activity against the two Gram positive bacteria *Streptococcus faecalis*, and *Staphylococcus aureus* and three Gram negative bacteria *Klebsiella pneumoniae*, .*E-coli*, and *Pseudomonas aeruginosa* .The growth of *S.faecalis, Klebsiella pneumonia* and *Pseudomonas aeruginosa* was strongly inhibited by the EtOH extract of fruit part of *S. suaveolens* which is shown by MIC value 250 µg/ml, 500 µg/ml and 500 µg/ml respectively. The remaining Gram positive and Gram negative bacteria showed almost no susceptibility growth.

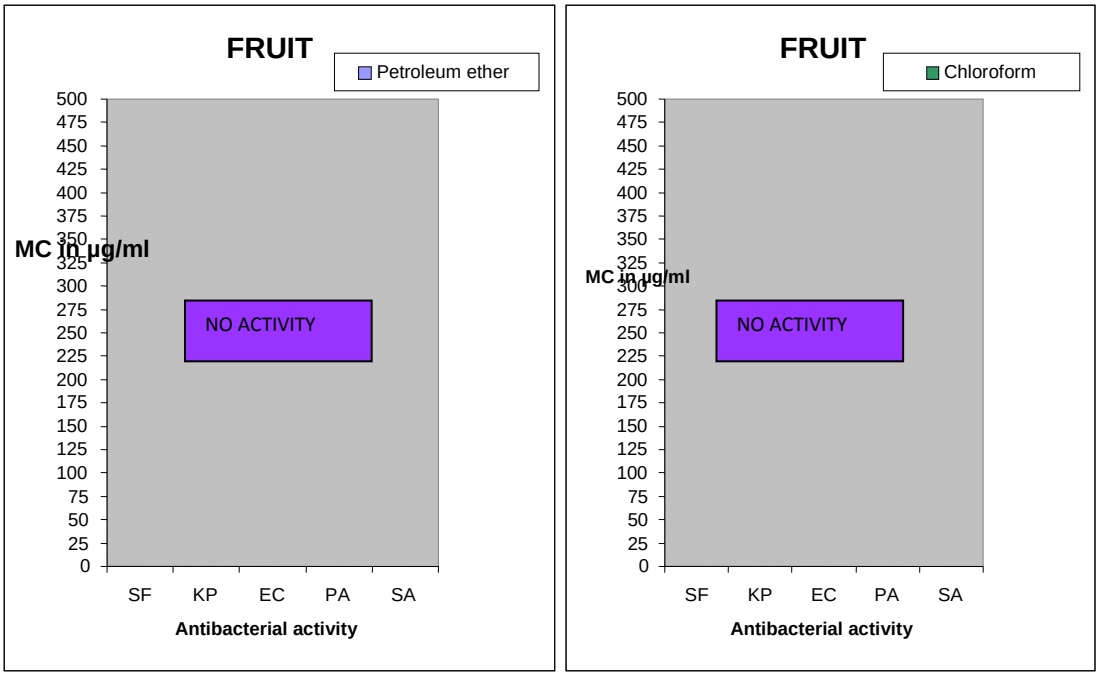
The Testing of antifungal activity of extracted in different four solvents of fruit part (Table 1 and Fig. no.2) exhibited large zone of inhibition toward the two fungi *Cryptocloccus neoformans* , *Sporothrix schenckii* and *Candida parapsilosis* which shown by MIC value 250 µg/ml, 250 µg/ml and 500 µg/ml respectively while in case of chloroform extract of fruit part exhibited large zones of inhibition toward the three fungi *Sporothrix schenckii, Trichophyton mentagrophytes* and *Candida parapsilosis* which shown by MIC value 250µg/ml, 250 µg/ml and 500 µg/ml respectively and EtOH extract of fruit part exhibited large zones of inhibition towards the five fungi *Candida albicans, Cryptocloccus neoformans, Sporothrix schenckii, Trichophyton mentagrophytes, Candida parapsilosis* which shown by MIC value 500µg/ml, 250 µg/ml, 500 µg/ml, 500 µg/ml and 500 µg/ml respectively **.**

Table 1: Antimicrobial activity of Fruit part Extract,

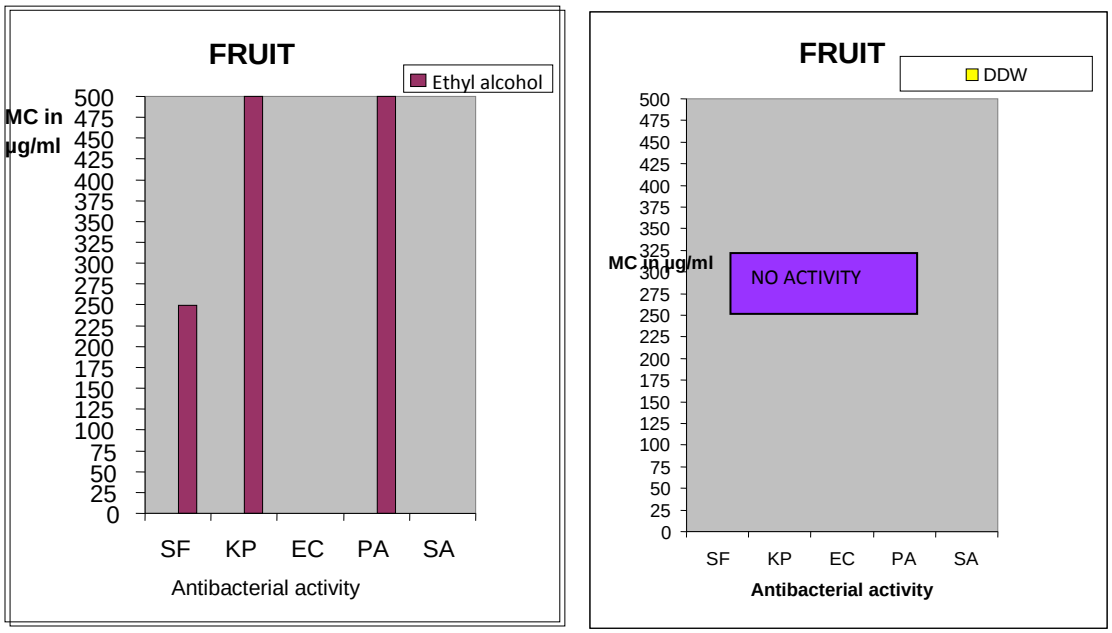
S.No.	Plant Part	Solvent	Minimum inhibitory conc. (MIC) in µg/ml against										
			BACTERIA					FUNGI					
			1	2	3	4	5	6	7	8	9	10	11
1.	Fruit	Petroleum ether	-	-	-	-	-	-	250	250	-	-	500
2.	Fruit	Chloroform	-	-	-	-	-	-	-	250	250	-	500
3.	Fruit	Ethyl alcohol	250	500	-	500	-	500	250	500	500	-	500
4.	Fruit	Double Distilled Water	-	-	-	-	-	-	-	-	-	-	-

1. Streptococcus faecalis (G+) , 2. Klebsiella pneumoniae (G-) , 3.E-coli (G-) , 4.Pseudomonas* aeruginosa *(G-) , 5. Staphylococcus aureus(G+).

****** 6.Candida albicans, 7. Cryptocloccus neoformans, 8. Sporothrix schenckii, 9. Trichophyton mentagrophytes, 10.Aspergillus fumigatus, 11.Candida parapsilosis.***



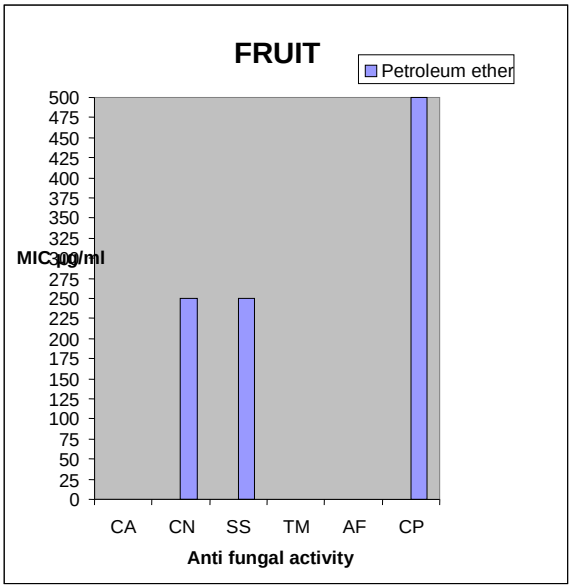
(a) **(b)**



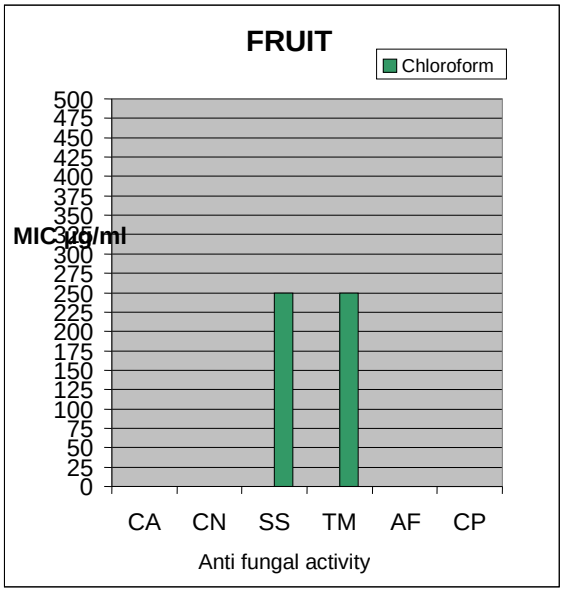
(b) **(d)**

SF=*Streptococcus faecalis* (G+), KP=*Klebsiella pneumoniae*(G-), EC=*E-coli*(G-), PA=*Pseudomonas aeruginosa*(G-),SA=*Staphylococcus aureus*(G+)

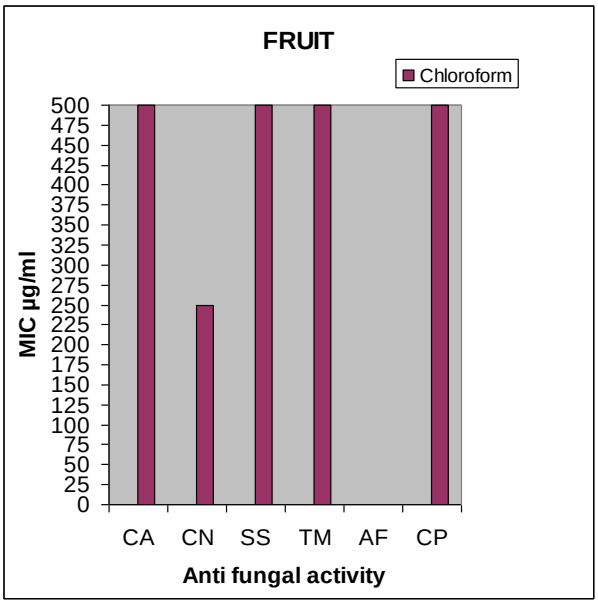
Fig.1: (a) Antibacterial activity of Fruit part extract in Petroleum ether solvent.
(b) Antibacterial activity of Fruit part extract in Chloroform solvent.
(c) Antibacterial activity of Fruit part extract in Ethyl alcohol solvent.
(d) Antibacterial activity of Fruit part extract in Double Distilled water solvent.

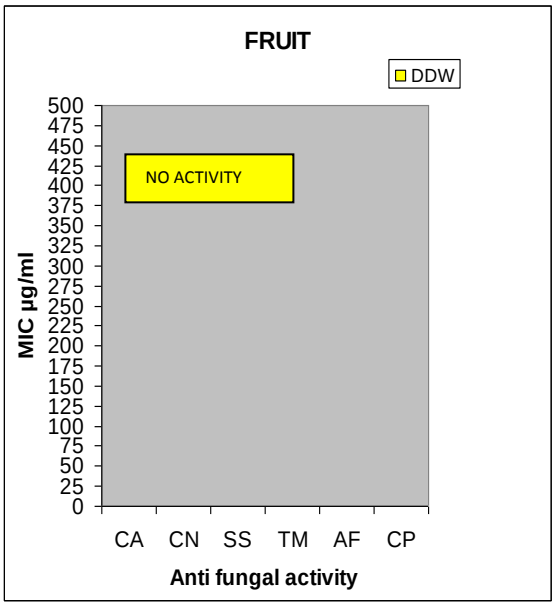


(a)



(b)





(d)

CA=*Candida albicans*, CN=*Cryptocloccus neoformans*, SS=*Sporothrix schenckii*, TM=*trichophyton mentagrophytes*, AF=*Aspergillus fumigatus*, *CP=Candida parapsilosis*

Fig.2: (a) Antifungal activity of Fruit part extract in Petroleum ether solvent.
(b) Antifungal activity of Fruit part extract in Chloroform solvent.
(c) Antifungal activity of Fruit part extract in Ethyl alcohol solvent.
(d) Antifungal activity of Fruit part extract in Double Distilled water solvent.

These preliminary results from the evaluation of the antibacterial and antifungal activities of the crude petroleum ether, $CHCl_3$ and EtOH extracts gave evidence of the presence of compounds of biological interest in the analyzed extracts. The plant species used in this investigation were selected

primarily on the basis of their folkloric medicinal properties and it is interesting that the exhibited antimicrobial activities correlated well with their traditional uses. Indeed , the plant *S. stereospermum* used as Anticancer, Diuretic , Inflammatory , Analgesic , Headache , Migraine , Hiccough, Leprosy , Healing of bone , Hemorrhage and Hyperacidity etc. The inhibition of *S.faecalis* (Gram + ve Bacteria) strains can also explain the effectiveness of Fruit extract in the prevention and treatment of above such disease and inhibition of *Cryptocloccus neoformans, Sporothrix schenckii* and. *Trichophyton mentagrophytes* can also explain the effectiveness of Fruit (Petroleum ether, $CHCl_3$ and EtOH) crude extract in the prevention and treatment of above such disease .The *Cryptococcus neoformans* a genus of fungi. It is pathogenic to man. It has a marked predilection for the Central Nervous System causing sub acute or chronic disease which exhibited large zone of EtOH curde extract of Fruit part have MIC value 250 - 500µg/ml (Table 1) above result clearly show that Fruit part have a some constituents which have great therapeutic potential towards Central Nervous System. Therefore, in the present study, attention has been paid regarding a systematic fractionation of the analyzed crude plant extracts, thus providing a basis for further studies focused on isolating the active constituents through a bioassay- guided isolation procedure.

Acknowledgement

Pleasure to express my deep sense of gratitude to my teacher and research guide ***Late* Dr. Kaushal Kumar**, ***Professor and Head, Department of Chemistry,*** Gurukula Kangri University, Haridwar, who introduced me to the fascinating area of research in traditional Ayurvedic system of medicine.

I would like to thank **Dr. C.M. Gupta,** ***Director*** (CDRI) Lucknow**,** for having given me a wonderful opportunity to carry out my Research work and Trainee in a renowned institute. I am highly indebted to **Dr. Chandan Singh**, ***Head Medicinal Chemistry Division*** (CDRI) Lucknow, **Dr. A.K Molik,** ***Ayurvedic Scientist*** Roorkee and **Dr. S.B Sharma,** ***Dean faculty of sciences motherhood University*** Roorkee, Haridwar for their help and cooperation. I offer my sincere thanks to **Dr. Anil K. Goel** ***(Senior Assistant Director)*** and **Dr. P.K. Shukla** ***(Department of Microbiology) Center Drug Research Institute,*** (CDRI) Lucknow**,** who has been a constant motivation for the my work. Last, but not the least, I would like to express my heartily thanks to all my Research work of ***CDRI, CIMAP, ITRC, NIPAR and IIT Roorkee***, for making me and indulged me in all the possible matters.

References

1. Kambu, K. **(1990)** Elements de phytotherapie compare: Planets Medicinales Africainales Africaines, Editions du CRP, Kinshara. Zaire.
2. ASTHANA,A. MALL, H.V. DIXIT, K. and GUPTA, S**.(1989)**. Int. J. Crude Drug. Res. 27:25-28.
3. Aswal BS, Goel AK, Screening of Inidan plants for biological activity : Part XV. *Indian J. Exp Biol.***1996** ; 34 : 444 – 467
4. National Committee for clinical laboratory standards, **1998**. references method for broth dilution antifungal susceptibility testing of conidium- forming filamentous fungi: proposed standard. Document M38-P. National Committee for Clinical Laboratory Standards, Wayne, PA.
5. Green T.W ; *J . Infect. Disease*, 74, 36 **(1944)**.
6. Marrow .G and Berry G. P ; J. Bat. , 38, 290 **(1938).**